

Lipids in *Euphausia superba*

by

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To Jacob

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Abstract

Aspects of the life history and overwintering survival strategies of Antarctic krill (*Euphausia superba*) were interpreted through analyses of lipid class, fatty acid and sterol content, and composition. Both laboratory and field studies were undertaken to provide information on the biochemical and physiological consequences of starvation, on dietary sources and on reproduction.

Starvation in krill, both short term (19 days) and long term (130 days), was investigated. Lipids are a source of short term energy in krill. The relative levels of polar lipids, free fatty acids and cholesterol in the digestive gland provide indices of the nutritional condition of *Euphausia superba* in the field in the short term. However, throughout long term starvation levels of both triacylglycerol and polar lipid in krill did not change significantly. Therefore, lipid metabolism is not considered to be the major mechanism for energy production during long term starvation as is usually the case in most other polar zooplankton.

Lipid profiles of krill fed on diets of *Phaeocystis pouchetii*, and of diets of diatoms were compared. Krill fed these two diets showed no significant differences in lipid levels. *Phaeocystis pouchetii*, although being deficient in a number of what are normally referred to as the essential fatty acids, was found to be nutritionally equivalent to diatoms as a food source for *Euphausia superba*. Krill may possess the ability to convert exogenous shorter chain fatty acids to the long chain essential polyunsaturated fatty acids, eicosapentaenoic (EPA, 20:5 ω 3) and docosahexaenoic (DHA, 22:6 ω 3); this hypothesis has also recently been proposed for penaeid prawns. The results from the present investigation of *Euphausia superba* suggest the ability to convert dietary derived fatty acids to EPA and DHA may be more widely spread than previously believed.

The use by krill of bacteria as a nutrient source was also investigated. Bacterial cultures isolated from the stomach and the digestive gland of krill contained strains able to produce polyunsaturated fatty acids including eicosapentaenoic acid (20:5 ω 3). These findings may partially explain the high

levels (approximately 50% of total fatty acids) of essential fatty acids found in the digestive gland of krill.

Lipid biomarkers in krill were investigated. It was found that sterols in the digestive gland indicate composition of the recent diet of krill. Sterols can be also be used to quantify dietary input from individual phytoplanktonic species. Fatty acids, like sterols, can be used as biomarkers to provide information on food sources of *Euphausia superba*.

The role of lipids in reproduction was investigated. Reproductive stores were determined in both male and female krill. High lipid levels were found in reproductive female krill and very low levels of lipid were found in male krill following reproduction. Despite male krill feeding actively during reproduction, the level of storage lipid (triacylglycerol) was depleted. Female krill regressed sexually after spawning and survived through to the following season. Male krill, however, died shortly after reproduction. Fewer males in the Antarctic krill population would reduce competition during the food-limited winter allowing resources to be preferentially invested in females.

The lipid profile of *Euphausia superba* was compared to that of a temperate euphausiid, *Nyctiphanes australis*. Seasonal comparisons between the polar and temperate species were made. The potential of the euphausiid, *Nyctiphanes australis* as an aquaculture feed in terms of lipid, pigment and fluoride content was investigated. Like *Euphausia superba*, *Nyctiphanes australis* contained high levels of the essential long chain polyunsaturated fatty acids (PUFA); EPA and DHA. Astaxanthin was the main carotenoid in *Nyctiphanes australis*. Both high PUFA and astaxanthin levels suggest this species could serve as a suitable feed for the aquaculture industry.

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Chapter 1. General Introduction

1.1 Antarctic Krill

'Krill' is a word originally applied by Norwegian whalers to the shrimp-like crustaceans which are extremely abundant in polar seas. Krill belong to the order *Euphausiacea* of which 85 species are known. Although there are seven species of euphausiids that live in Antarctic waters, the term Antarctic krill usually refers to the dominant species, *Euphausia superba* (Appendix 1).

Euphausia superba are of considerable importance in the Antarctic marine ecosystem. Krill are one of the prime converters of phytoplankton and hence most Antarctic marine vertebrates either depend on krill directly or through a close trophic association (Figure 1.1). The importance of krill in the economy of the Southern Ocean was expressed by Marr (1962) during the *Discovery* Investigations. "The astronomical abundance in which it exists and the key position it holds in the industry and ecology of the Antarctic seas is revealed by a review of the vast and catholic multitude of its predators".

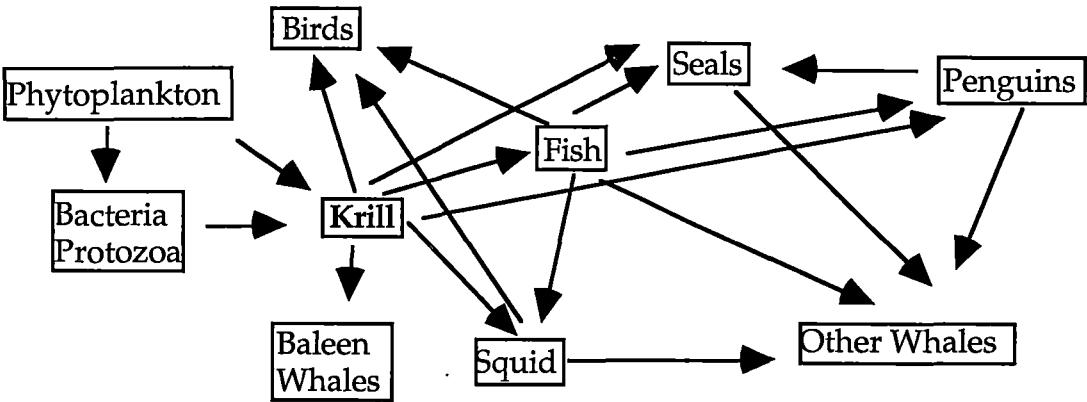


Figure 1.1 A simplified scheme of the Antarctic marine food web.

In addition to this multispecies dependency, krill are also commercially harvested for both human and animal feed primarily by Chile, Japan, Ukraine, Poland and Russia. The Soviets started fishing krill in the early 1960s and reported vast schools that "turned the ocean red" (Ross and Quetin 1988). To determine just how vast schools of krill are is proving difficult. Although Antarctic krill have been investigated intensely, there are still major discrepancies in our understanding of the life history of this species.

Commercial krill catches have been as high as $5,3 \times 10^5$ tons in 1981/82, however, catches have declined considerably with only $8,4 \times 10^3$ tons being caught in 1993/94 (Miller 1991, Anon 1995). Based on present biomass estimates and catch levels, the krill fishery has not reached potential harvesting capacity (Miller 1991). A management body to regulate the krill harvest was established in 1980 as a result of both concerns of over-harvesting, and the historical exploitation of Antarctic marine resources (Nicol 1990). Due to the critical position of krill in the Southern Ocean ecosystem, the associated ecological costs of over-harvesting would be devastating. Confident biomass and production estimates must be used to manage the krill fishery as a sustainable resource.

1.1.1 Krill Biomass

One of the major difficulties in estimating krill biomass is due to the sheer size of the Southern Ocean and the associated seasonal fluctuations in ice cover. Large scale extrapolations of commercial catch data and krill survey results must be made in estimating krill abundance. *Euphausia superba* have a circumpolar distribution which is restricted to the north by the Antarctic Polar Front. Distribution is thought to be influenced by large-scale oceanic circulation patterns in this region. Information on early summer and winter distribution is scarce as most of the area to the south of the front is under pack ice during this time.

Estimating standing stocks is inherently problematic due to the patchy distribution of krill. Krill commonly swarm. Krill swarms can range from several meters to several kilometres in length. Generally swarms are dense with acoustic estimates of concentrations up to $35,000 \text{ animals m}^{-3}$ (Klindt and Zwack 1984). Many factors such as improved foraging efficiency,

enhanced reproductive success and evasion of predation have been implicated in swarming behaviour (reviewed by Miller and Hampton 1989). Due to the huge variations in size of these krill aggregations, the task of assessing biomass using both catch data and acoustic surveys is difficult. Reported estimates of standing stocks of krill, range from between 55 to 7000 million tonnes (Miller and Hampton 1989).

1.1.2 Krill Production

Fundamental gaps in our knowledge of the basic krill biology and physiology makes estimating krill production difficult. To estimate krill production, parameters such as growth rate, mortality and reproduction must be fully described. Traditional length frequency analysis cannot be applied because morphometric characteristics of krill are not linear with age. During times of limited food supply (which can be up to 8 months of the year), growth rates of krill have been reported to be negative (Ikeda and Dixon 1982, Ettershank 1983, Quetin and Ross 1991). This makes determining krill growth rates and longevity inherently difficult. Although there is conflicting evidence for the existence of overwinter lipid stores (Hagen 1988, Quetin et al. 1993, Clarke 1984), laboratory studies have shown that krill utilise body protein as a source of metabolic energy during starvation. As they continue to moult they decrease in body size (Ikeda and Dixon 1984).

Accompanying negative growth, external sexual characteristics of mature krill have been shown to regress to those of their immature forms (Ikeda and Dixon 1982). Hence growth rate estimates and life span determination based on state of maturity can be deceptive. Although much research has been focused on krill life span, estimates range between 2-11 years (Ikeda et al. 1985). Various methods have been used to estimate krill longevity with limited success. Fluorescent pigments, known as lipofuscins, are thought to be the waste products of oxidative metabolism. Lipofuscins accumulate with age and linear correlations have been found between concentration of these pigments and age in short lived animals. Lipofuscins have been used to age krill (Ettershank 1983), however, considerable variability in results has indicated the need for a reassessment of the technique on methodological and technical grounds (Nicol 1987, Nicol 1991).

Natural mortality rate is a function of age and estimates also vary widely. The causes of mortality in krill (excluding predation) are not well understood. Mortality in the winter as a result of environmental stress is not known. Mortality associated with reproduction has been reported in other euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa raschi* (Mauchline 1960, Mauchline 1966). However, conflicting evidence exists for breeding related mortality in *Euphausia superba* (Marr 1962, Quetin and Ross 1984, Watkins et al. 1986).

In estimating production, one of the many parameters involved in the formulation of an energy budget includes the cost of reproduction. To date all reproductive costs in krill have been associated with egg production and seasonal fecundity (Miller and Hampton 1989). Costs of male reproduction is considered insignificant, however, nothing is known of the energy associated with sperm production and transfer (Quetin et al. 1994).

1.1.3 Chemical composition of krill

The chemical composition of krill has been widely researched in view of its commercial use for both human and animal consumption. Krill are nutritious and have a sweet taste which is similar to that of shrimp. Frozen and dried krill are used for animal food and peeled tail meats are used for human consumption. On average krill contain 78 - 83% water, 12 - 15% crude protein, about 2% chitin and glucides and 3% ash (Grantham 1977, Suzuki and Shibata 1990). Krill have large amounts of vitamins A and E and a high concentration of the pigment, astaxanthin (Funk and Hobson 1991).

Many studies have been conducted on the lipid content of *Euphausia superba*. Reported levels of total lipid range from 6-43% dry weight depending on life stage, sexual maturity, temporal and spatial variations (Ferguson and Raymont 1974, Clarke 1984, Saether et al. 1985, Reinhardt and Van Vleet 1986). Lipid class and fatty acid composition of krill have also been well characterised. Krill generally have high levels of polyunsaturated fatty acids which are essential nutrients for most animals. Despite the array lipid studies on *Euphausia superba* little is known on the physiological role of lipids in this species. The pathways of lipid catabolism in krill are unknown. Conflicting evidence exists on whether krill actually store lipid reserves and if so, in

what form they are stored. This kind of information is essential in considering the basic life history of a species.

With the high levels of proteins, polyunsaturated fatty acids and vitamins, krill is an ideal food for most animals. One major draw back of using krill as an animal feed is it's high fluoride content. Levels of fluoride in *Euphausia superba* have been found to be unacceptable for either human consumption or for stock feed (Budzinski et al. 1985, Nicol and Stolp 1989). Fish, on the other hand, accumulate fluoride in their skeleton only and fish flesh is unaffected by fluoride content of the food source (Oehlenschläger and Manthey 1982). Hence, as a fish feed krill are considered ideal.

The commercial interest in krill has long been established. As krill are of immense importance to the Antarctic marine ecosystem, relevant data necessary for scientific management of this crustacean fishery is imperative. It is difficult to determine the productivity of Antarctic krill based on available biological data. This study has investigated several aspects of krill life history. Lipids have been used as a primary investigative tool in this study because they are known to be particularly useful as biochemical probes in biological systems. In the following sections background is provided on the lipid classes examined in this study, and their use in food chain studies.

1.2 Lipids

Lipids are a diverse group of compounds. They are of great nutritional and reproductive significance in all living organisms. The major lipid classes include triacylglycerols, phospholipids, glycolipids, sterols and hydrocarbons. Lipids are used in physiological and biochemical studies because they are important both as energy reserves and as structural components of the cell. In food chain studies lipids can be used as chemical signatures or biomarkers that identify the presence of certain organisms or groups of organisms.

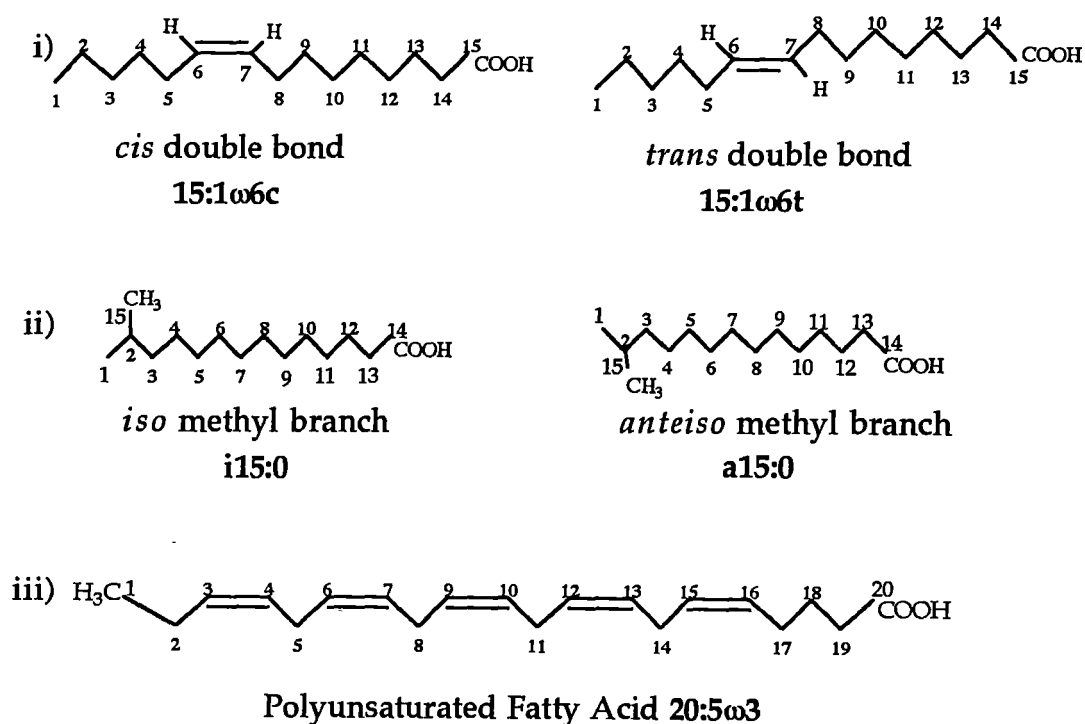
On a weight for weight basis, lipids liberate double the energy content (9.5 kcal/gram) compared to proteins (4.4 kcal/gram) and carbohydrates (4.2 kcal/gram) (Fessenden and Fessenden 1982). Lipid is the ideal medium if large energy storage is required by an organism, as it requires the least mass. In the polar marine environment, where food is abundant for a few months and scarce for the remainder of the year, in most organisms the ability to store lipids is their currency for survival. In addition to the high energy content, lipids have a low specific gravity which provides buoyancy in pelagic marine organisms (Hagen 1988).

Antarctic phytoplankton incorporate as much as 80% of their fixed carbon into lipid as compared to 20% incorporation in temperate phytoplankton (Smith and Morris 1980). The energy of the summer phytoplankton production is conserved in lipid reserves in the herbivorous crustaceans in Antarctica (Hagen 1988). These reserves are in turn the vital link to winter survival for the many dependant predators. Due to the absence of wax ester storage, there has been much speculation as to whether krill use lipid as a source of energy in the winter (Clarke 1980, Hagen 1988, Quetin et al 1994).

Littlepage (1964) was the first to demonstrate that polar euphausiids were richer in lipids than those from warmer waters and suggested that this may be a general trend for all polar marine invertebrates. Hagen (1988) also noted that the abundance of lipids found in Antarctic herbivorous crustaceans was not reflected in temperate or tropical crustaceans. The high lipid content in polar crustaceans is thought to be a compensation for extreme seasonality of primary production rather than a temperature adaptation (Clarke 1983, Hagen 1988).

1.2.1 Fatty Acids

Fatty acids are the essential building blocks of many of the complex lipids. Most fatty acids in plants and animals contain between 12 to 32 carbon atoms per molecule. Fatty acids are usually straight chain but can be branched or cyclic. They are either saturated (containing no double bonds) or unsaturated (containing one to six double bonds) and contain a terminal methyl group and a terminal carboxylic acid. Double bonds have a *cis* or *trans* geometric configuration (Figure 1.2 (i)) which affects the melting point of the compound. Branched fatty acids can occur in the *iso* or *anteiso* configuration (Figure 1.2 (ii)) which can be of taxonomic significance in some bacteria. Fatty acids with more than two double bonds are referred to as polyunsaturated fatty acids (PUFA) (Figure 1.2 (iii)). All double bonds in PUFA are methylene interrupted.



20 : number of carbon atoms

5 : number of double bonds

ω 3 : position of the first double bond from the methyl end

Figure 1.2 Fatty acid structures.

Fatty acids are important components of the cell and organelle membrane. The fluidity of biomembranes is a function of the degree of unsaturation of its constituent fatty acids. The degree of unsaturation present in membrane lipids is thought to be directly related to temperature. Unsaturated fatty acids have a lower melting point than saturated fatty acids. The more double bonds in fatty acids within the membrane, the more fluid the cell membrane. The problem of maintaining membrane fluidity (and the associated metabolic processes) is more acute in organisms in polar waters than in temperate and tropical waters. Polyunsaturated fatty acids are therefore of particular significance in organisms living in cold waters. It has been suggested that the phospholipids of Antarctic plankton contain a higher degree of unsaturation in order to maintain membrane fluidity at low temperatures (Clarke 1983). The seasonality of lipid storage in euphausiids is believed to be the key to understanding differences in lipid content and composition. However, few comprehensive seasonal studies on lipids of euphausiids have been performed.

Polyunsaturated fatty acids are generally thought to be only synthesised *de novo* by photosynthetic organisms (Sargent and Whittle 1981) and are therefore considered essential to heterotrophic organisms. PUFA of the linolenic ($\omega 3$) and the linoleic ($\omega 6$) families have been recognised as very important nutrients for the growth of crustaceans. It has been suggested that crustaceans must derive these nutrients exclusively from their diet (Sargent and Whittle 1981, D'Abramo and Shyn-Shin 1993). Polar herbivorous crustaceans are rich in the $\omega 3$ fatty acids, especially 20:5 $\omega 3$ (eicosapentaenoic acid) and 22:6 $\omega 3$ (docosahexaenoic acid) as dictated by the high PUFA levels in the phytoplankton on which they feed. The levels of PUFA and in fact the fatty acid profiles of phytoplankton vary between species. Fatty acids can be used to determine quantity and composition of food sources, because zooplankton can reflect, to a certain degree, the fatty acid profile of their diet (Sargent and Whittle 1981, Saether 1986).

1.2.2 Triacylglycerols

Triacylglycerols are neutral lipids which contain two unit molecules: glycerol and fatty acids (Figure 1.3).

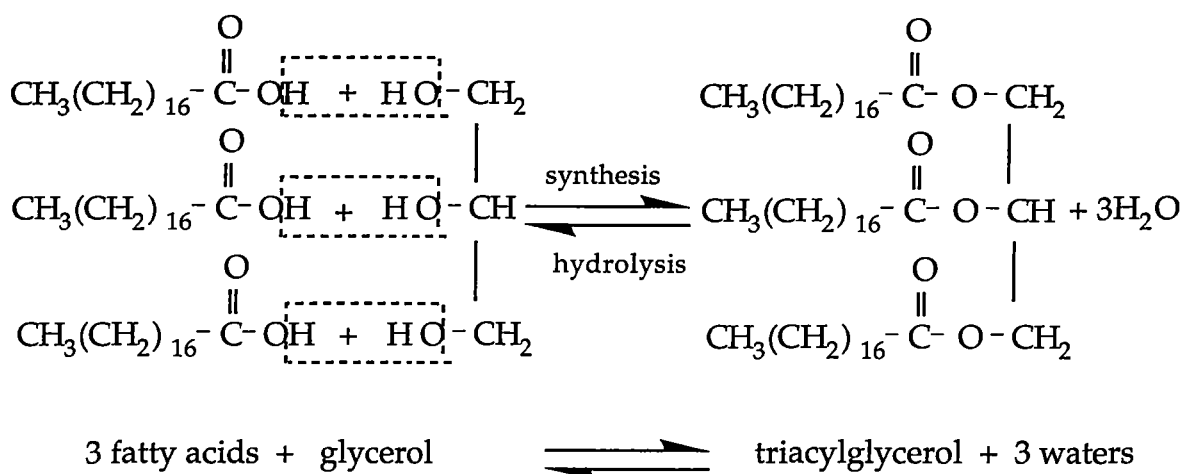


Figure 1.3 The synthesis and hydrolysis of triacylglycerol.

Fatty acids are released upon hydrolysis of triacylglycerols (Figure 1.3). When an organism ingests triacylglycerols they are hydrolysed into monoglycerides, diglycerides, glycerol and fatty acids which are absorbed through the intestinal wall (Fessenden and Fessenden 1982). These products are then: (1) used to synthesise the organisms own lipids or other compounds, and (2) metabolised for energy. The major site for lipid storage and synthesis in crustaceans is thought to be the digestive gland (Teshima and Kanazawa 1980, Dall et al. 1992). Triacylglycerol is the major deposit of storage lipid in all animal cells (Lehninger 1975) and it represents an important energy reserve in crustaceans. Generally, triacylglycerol is accumulated during feeding and depleted during starvation. As triacylglycerol content is directly affected by exogenous food intake it is an ideal lipid class to use as an index of biochemical condition (Fraser 1989). The percentage triacylglycerol, as a function of the total lipid of an organism, therefore can be used to assess it's physiological status (Fraser 1989).

1.2.3 Phospholipids

Phospholipids (Figure 1.4) are amphoteric in nature and thus form the infrastructure of biomembranes. They are essentially constructed in the same fashion as triacylglycerol except that in the place of the third fatty acid there is a phosphate group (or grouping that may contain both phosphate and nitrogen).

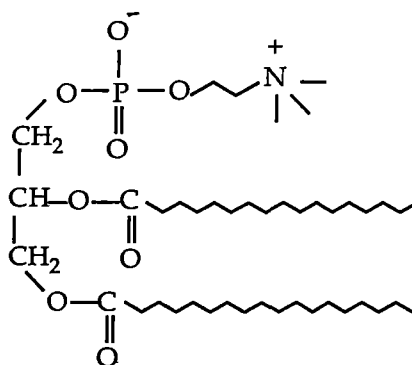


Figure 1.4 Structure of phospholipid (phosphatidyl choline).

The phosphate group can be ionised and hence is soluble in water while the long fatty acid chain is hydrophobic. These molecules are arranged in a bilayer with the nonpolar alkyl chains of the fatty acids in the centre and the polar phosphate end orientated on the surface of the cell membrane. Transfer of nutrients and metabolic products across the membrane is thus facilitated. Membrane fluidity is vital to achieving this transfer of nutrients so a high concentration of PUFA can be found in the phospholipids of membranes.

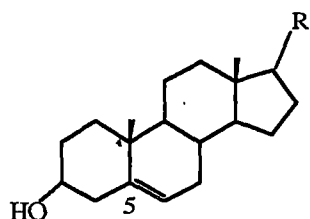
In most organisms phospholipids are used mainly as structural elements in the plasma membranes. However, polar euphausiids have been reported to utilise phospholipid as well as triacylglycerol and wax ester as storage material (Ellingsen 1982, Hagen 1988). Phytoplanktonic PUFA can be deposited unchanged in both the phospholipids and neutral lipids of invertebrates (Sargent and Whittle 1981). In addition to the analysis of triacylglycerol, the physiological status and the dietary components of an organism can be investigated using phospholipids.

1.2.4 Sterols

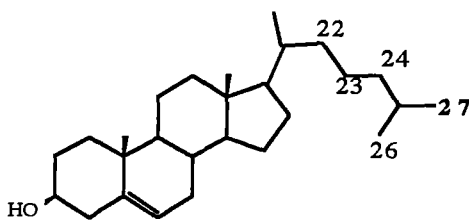
Sterols are compounds containing a four ring system (Figure 1.5 (i)).

Cholesterol is the major sterol found in animals (Figure 1.5 (ii)) and it is the precursor of steroid hormones such as progesterone, testosterone and cortisol. Sterols are components of all eucaryotic plasma membranes (Stryer 1988).

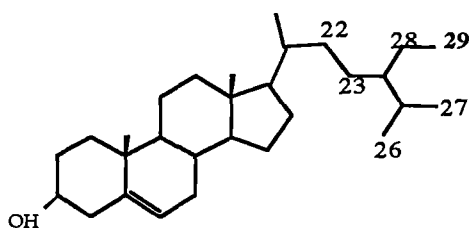
Plants have a wide range of sterols which are related in structure to cholesterol. The numerous arrangements of the side chains of the sterol ring system (Figure 1.5 (ii-vi)) are responsible for the range of sterols found in marine phytoplankton. Knowledge of this range of sterols can provide information about algal taxonomy and the species composition of field samples.



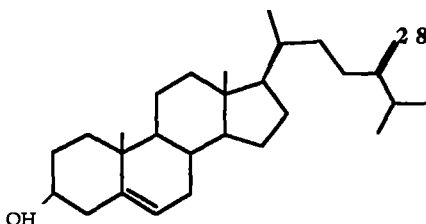
i) Basic Δ^5 sterol structure



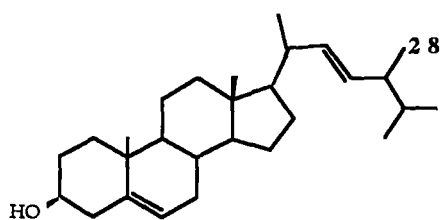
ii) Cholesterol (C_{27} sterol)



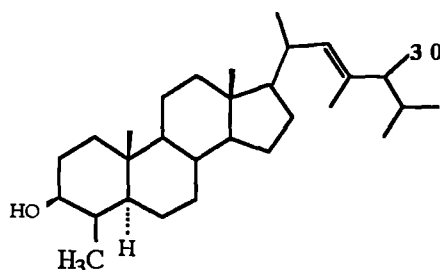
iii) 24-Ethylcholesterol (C_{29} sterol)



iv) 24-Methylenecholesterol (C_{28} sterol)



v) Brassicasterol (C_{28} sterol)



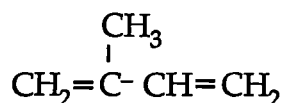
vi) Dinosterol (C_{30} sterol)

Figure 1.5 Sterol structures.

The majority of marine organisms rely on phytoplankton as their sole source of sterols, directly in the herbivores and indirectly for the carnivores (Ballantine et al. 1979). Marine crustaceans cannot synthesise sterols *de novo* however, they are able to dealkylate phytosterols which are then converted to cholesterol (Teshima 1982).

1.2.5 Hydrocarbons and pigments

Hydrocarbons, as the name implies, are a class of compounds containing hydrogen and carbon which are formed by biosynthetic processes in both marine and land organisms. Hydrocarbons can vary in molecular weight, degree of saturation and polarity. A remarkable array of compounds are formed from the linkage of the basic isoprenoid (C₅) unit:



Plants absorb light due to the alternating double and single bonds of the isoprenoid units in pigment compounds (Stryer 1988). A wide range of photosynthetic pigments and other isoprenoid alkanes are present in phytoplankton (Figures 1.6 and 1.7) and can be species specific. Measurement of these isoprenoid biomarker compounds can be used to determine algal classes present in phytoplankton populations.

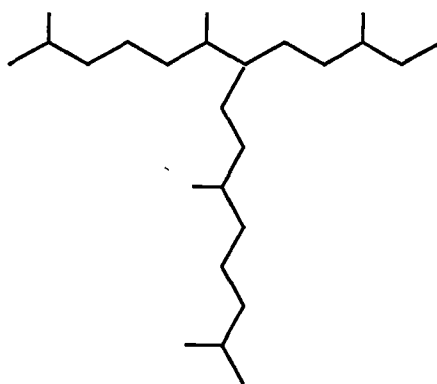


Figure 1.6 Structure of a C₂₅ isoprenoid alkane skeleton found in marine algae.

The most spectacular of pigments are the vivid red carotenoids (C₄₀ isoprenoid hydrocarbons) which are found widely among invertebrates, particularly in Crustacea. The carotenoids such as astaxanthin and canthaxanthin, (Figure 1.7) which are found in marine organisms such as krill and salmonids, are derived from dietary plant carotenes and xanthophylls. Their major metabolic role is that of photoprotection (Hairston 1979), and are also used as antioxidants and as precursors to vitamin A (Goodwin 1984). These pigments cannot be biosynthesised by aquatic animals, they must be assimilated from the diet. In the aquaculture industry there is a high demand for these carotenoids as feed supplements, particularly in the rearing of salmonids where the maintenance of its pink flesh is of high commercial importance (Storebakken 1988). Krill, being high in astaxanthin levels, are therefore a potential component for aquaculture feeds (Shimizu et al. 1990).

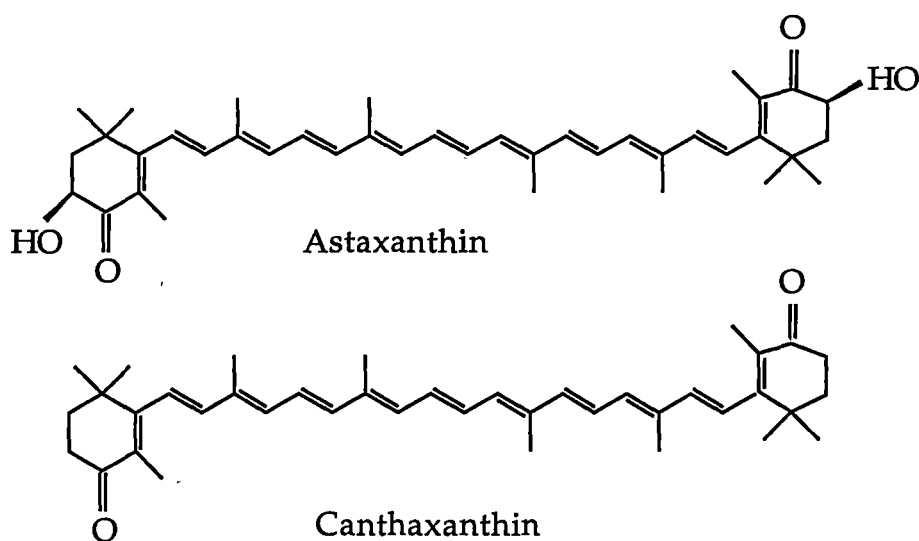


Figure 1.7 Carotenoid pigments found in krill.

1.2.6 Lipid biomarkers

Various fatty acids, sterols, and hydrocarbons that move unaltered through the trophic chain can be used as biomarkers. Both fatty acids and sterols are of interest as biomarkers because they can be markedly species specific (Sargent and Whittle 1981). Hydrocarbons are considered metabolically inert, hence dietary derived hydrocarbons generally are not altered through the food web (Mackie et al. 1974).

Biomarker distributions can be used to understand trophic relationships between organisms, from bacteria to mammals. The presence of lipid compounds in organisms can be adequately species specific so as to be useful in taxonomic classification. In addition to their chemotaxonomic uses, biomarkers can provide insight into the biochemical and geochemical cycling of organic matter (Laureillard and Saliot 1993). Many lipid biomarkers are resistant to degradation. These organic compounds can be preserved over extended periods in sediments and also may be useful in paleoenvironmental studies (Sikes and Volkman 1993).

Fatty acids derived from the phospholipids of microorganisms associated with sediments provide reproducible and quantitative measures of the biomass and community structure of microbial assemblies (e.g. White 1983, Guckert 1985). For example, *iso* and *anteiso* branched fatty acids are characteristic of bacteria, hence their presence in higher organisms can be used to identify bacterial association.

Sterols can be used as chemical markers for distinguishing algal classes, and in some cases, specific algal species. 24-Methylcholesta-5,22E-dien-3 β -ol (Figure 1.5 (iv)) is a characteristic sterol in diatoms (Patterson 1987). More specifically 23,24-dimethylcholesta-5,22E-dien-3 β -ol has been found to be a major sterol in the pennate diatom, *Fragilaria pinnata* (Barrett 1995). The authors suggest this sterol may be used as a biomarker for *Fragilaria pinnata*. Dinosterol (4,23,24-trimethyl-5 α -cholest-22E-en-3 β -ol) (Figure 1.5 (vi)) has long been recognised as a marker for dinoflagellates. Although it is not widespread, it may occur in other algae (e.g. Nichols et al. 1990).

As most invertebrates cannot synthesise sterols they must acquire them via a dietary source. The major sterol in crustaceans is cholesterol (Figure 1.5 (ii))

which can be derived through the conversion of phytosterols. Phytosterols are of interest in food chain studies because in addition to providing information on the recent diet of a grazer, rates of sterol conversion can be determined.

Fatty acids in phytoplankton can prove useful taxonomic tools. Fatty acid profiles in Antarctic diatoms show high levels of 20:5 ω 3, 16:1 ω 7c, 18:1 ω 7c and 18:1 ω 9c (e.g. Gillan et al. 1981, Nichols et al. 1986a). Although these compounds are not specific to diatoms, this particular fatty acid profile can be used to distinguish diatoms from another dominant algal species in Antarctic waters, the prymnesiophyte *Phaeocystis pouchetii*. *Phaeocystis* has very low levels of 20:5 ω 3 and is high in both 18:4 ω 3 and 18:5 ω 3 (Sargent et al. 1985, Nichols et al. 1991). 18:4 ω 3 can be a prominent component of the fatty acids in the calanoid copepod found feeding on *Phaeocystis* in Norwegian waters. In turn, 18:4 ω 3 has been found to be the prominent fatty acid in herring and sand eels which were feeding on the copepods in this region (Sargent and Henderson 1987).

Different species of krill can have distinct fatty acid profiles which provide information on feeding grounds and food sources of predators higher up the food chain. Analysis of lipids from finwhales feeding on krill (*Meganyctiphanes norvegica*) in Nova Scotian waters showed profiles high in 20:1 and 22:1 compared to lipids from finwhales feeding on *Euphausia superba* which are low in these fatty acids (Ackman and Eaton 1966). Fatty acids in milk samples of lactating Antarctic fur seals taken during foraging trips were analysed. The characteristic fatty acid pattern of the specific diatom diet upon which krill (*Euphausia superba*) had been feeding was evident in the fur seal milk (Iverson 1993).

Sterol biomarkers, in conjunction with fatty acids and other lipid compounds, can be used as powerful tools for biological studies. The use of biomarkers is a complementary technique or potentially a viable alternative to tedious microscopic methods used to determine the presence of organisms in gut samples, sediments and in the water column.

1.5 Objectives of this investigation

This study has attempted to gain an insight into several aspects of krill life history using lipids as the main investigative tool. Although the following chapters are distinct independent studies they are connected by a central theme. Using the various individual studies that this thesis comprises, a lipid based model was developed. The model is driven by the fundamental input into the system, being food. How the availability of food, in terms of both the quantity and quality of lipid, influences the survival of krill over winter. The extent of lipid expenditure on reproduction is incorporated into the model including implications on resource appropriation. Techniques incorporating thin layer chromatography - using a flame ionisation detector (TLC-FID), gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) have allowed the quantitative and qualitative determination of lipid data.

Because krill are inaccessible to study *in situ* for a substantial part of the year, laboratory starvation studies were undertaken to investigate how krill might respond physiologically to a limited winter food supply. A better understanding of overwintering mechanisms in relation to food supply is fundamental to our knowledge of growth and the aging process of krill. Several questions were posed: Are lipids a source of metabolic energy during starvation (both short and long term)? What is the rate of depletion of energy reserves during starvation? What is the role of the digestive gland in krill during starvation? By how much do krill shrink during starvation and are there differences between sexes? Do both male and female sexually regress at the same rate?

Growth is directly affected by both the quantity and quality of nutrients in the food source. Little is known about the nutritional characteristics of feed or alternative dietary sources for krill. When food is limited how do krill satisfy PUFA requirements? Krill were fed controlled diets in the laboratory to investigate the incorporation of dietary lipid. The nutritional value of *Phaeocystis pouchetii*, the diatom *Phaeodactylum tricornutum*, and bacteria as food sources were examined. Fatty acid and sterol biomarkers were used to investigate their usefulness in identifying and measuring phytoplanktonic dietary input.

The cost of reproduction is a major component of the energy budget in krill. The reproductive investment in male krill was investigated in this study. Lipid classes were used to assess the physiological status of male reproductive krill. Male mortality rate during reproduction was determined and compared to the rate found in female and non-reproductive krill.

Lipids in the temperate euphausiid *Nyctiphanes australis* were analysed with a two-fold purpose. Winter sampling of pelagic organisms in Antarctica is often not practical. Therefore a seasonal study of the lipid content and composition of *Nyctiphanes australis* was conducted and the results were compared to lipid data obtained for samples of *Euphausia superba* collected during the austral summer. This data was also used in an applied context. As the demand for aquaculture fishmeal is increasing dramatically the focus on krill, both local and further afield, is intensifying. The lipid, pigment and fluoride content of *Nyctiphanes australis* were determined as an appraisal of it's possible commercial use in aquaculture feed formulations.

**Chapter 2. Dietary related mechanisms of survival in .
Euphausia superba: biochemical changes during long term
starvation and bacteria as a possible source of nutrition.
Published in: Proceedings from the SCAR VI Biological
Symposium. B. Battaglia, J. Valencia, D. W. H. Walton,
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Co-authors: P. D. Nichols and S. Nicol.**

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bacteria as a possible source of nutrition, *Antarctic Communities Species,
Structure and Survival* pp. 194-201. ISBN 0521480337

Chapter 3. Changes in the digestive gland of *Euphausia superba* during short-term starvation: lipid class, fatty acid and sterol content and composition.

Published in: Marine Biology, 1993, 117, 441-448.

Co-authors: S. Nicol and P. D. Nichols.

3.1 Abstract

During a period of short-term starvation total lipid in the digestive gland of *Euphausia superba* Dana decreased from 21 to 9% (dry weight) throughout a 19 Day starvation period. Total lipid per digestive gland decreased significantly during starvation when compared to Day 0 animals, falling from 1960 (± 172) to 385 (± 81) μg . Polar lipid was the major lipid class utilised during starvation falling from 1510 (± 225) to 177 (± 46) μg per digestive gland (76 to 45%). Absolute levels of triacylglycerol fell from 300 (± 41) to 76 (± 5) μg , however relative levels remained unchanged. The relative level of free fatty acid increased significantly with starvation (4% to 39%) with absolute levels ranging from 79 (± 1) to 156 (± 20) μg per digestive gland. Absolute levels of all fatty acids per digestive gland declined continually until the end of the starvation period. The long chain polyunsaturated acids; eicosapentaenoic (20:5 ω 3) and docosahexaenoic (22:6 ω 3), decreased with starvation from 37 to 26% and 15 to 10% respectively whereas the saturated fatty acid, palmitic acid (16:0), increased from 15 to 22%. Cholesterol, the major sterol in this organ, increased from 17 (± 20) to 44 (± 13) μg per digestive gland on Day 3 and by Day 19 had returned to levels found in the digestive gland of Day 0 animals. Desmosterol followed a similar pattern to cholesterol increasing from 3 (± 1) μg per digestive gland on Day 0 to 11 (± 4) μg on Day 3 and falling to 2 (± 1) μg on Day 19. Other sterols in the digestive gland, predominantly of algal origin, fell from the levels found in the Day 0

animals to near zero amounts by Day 6. The digestive gland of *Euphausia superba* plays a dynamic role during short-term starvation in terms of lipid content and composition. The relative levels of polar lipids, free fatty acids and cholesterol in the digestive gland may provide reliable indices of the nutritional condition of *Euphausia superba* in the field. Sterols in the digestive gland are indicative of recent dietary composition of krill and may also be used to quantify dietary input from individual phytoplanktonic species.

3.2 Introduction

Antarctic krill are subjected to a marked seasonality in phytoplankton production which may result in limited food availability for up to eight months of the year, but with excess during the spring bloom conditions. *Euphausia superba* have been reported as having an extremely high tolerance to starvation and are able to survive up to seven months without food in the laboratory (Ikeda and Dixon 1982). To date overwintering mechanisms of *Euphausia superba* are still rather speculative due to the lack of adequate winter sampling. Several hypotheses have been put forth to interpret survival strategies during these periods of reduced phytoplankton abundance. Conflicting evidence has been reported on overwintering lipid stores, however recent reports suggest that krill utilise lipid reserves, mainly triacylglycerols, as an energy source in winter (Hagen 1988, Quetin et al. 1994).

The pathways of lipid catabolism during starvation in *Euphausia superba* are unknown. The major site of lipid storage and synthesis in crustaceans is thought to be the digestive gland or midgut gland (Teshima and Kanazawa 1980, Dall et al. 1992). This organ is also reported as being the principal site of absorption of digested food and storage of metabolites (Dall and Moriarty 1983) and marked changes occur in the fatty acids of the digestive gland of the tiger prawn *Penaeus esculentus* during starvation (Dall et al. 1992). If the role of the digestive gland in *Euphausia superba* is similar to that of other crustaceans, then dietary effects such starvation would be most evident in this organ.

The aim of this study was to determine the lipid class, fatty acid and sterol profiles of the digestive gland of *Euphausia superba* immediately after capture and during a subsequent short-term period of starvation. The results of these analyses will provide information on the role of the digestive gland during short-term starvation. Knowledge of the lipid composition of the digestive gland also may provide insight on how krill respond physiologically to a reduction in phytoplankton during winter.

3.3 Materials and Methods

Specimens of *Euphausia superba* were caught on February 2nd, 1991 in the Prydz Bay region of Antarctica 65°30.00 S and 77°59.00 E (Figure 2.1) in the top 20 meters of the water column with an RMT 8 net. Krill were subsequently maintained in darkness in a cold room ($0 \pm 0.5^{\circ}\text{C}$) on board RSV Aurora Australis for a 19 day period. Krill were kept in a 90 litre plastic container with sea water filtered through a 0.45μ Millipore filter. Krill samples were taken every three days during the starvation period (days 0, 3, 6, 9, 12, 15, 19). Carapace lengths were measured and digestive glands were dissected out and frozen in liquid nitrogen. Recent feeding was evident by the dark green colour of the gut contents of freshly caught krill which were designated the Day 0 animals (the day of capture). Samples were stored for 2-12 months in liquid nitrogen prior to extraction. Three analyses of batched digestive glands were performed for each day sampled using a total of seven digestive gland per day (three, two and two digestive glands per analysis). Krill used for this experiment were all of similar size with the daily mean carapace length between 14.75 and 15.75 mm. Three additional digestive glands for each day were freeze dried and weighed individually to determine water content. The average of these three dry weights per day was used to convert lipid, lipid class, fatty acid and sterol wet weight to their dry weight equivalence. Digestive gland weights were assumed to be equal and individual data reported is a third or a half of the batched sample wet weight. Procedures for lipid extraction and analysis are the same as those described in Chapter 2.

3.4.1 Statistics

A one factor analysis of variance was performed for total lipid, lipid class and sterol data for animals on each day of the starvation period compared to the Day 0 animals. Fisher's PLSD multiple comparison test was used and results reported using a significance level of 99%. Percentage fatty acid data were treated by both a correlation matrix analysis and least squares regression analysis using the Statistical Analysis System (SAS Version 6.03, 1988). All data are reported using \pm standard error.

3.4 Results

3.4.1 Lipid composition and content

Total lipid content decreased throughout the starvation period as would be expected resulting from a significant decrease ($p < 0.01$) in the dry weight of the digestive gland itself from 9.7 (± 1.2) mg on Day 0 to 4.3 (± 0.8) on Day 19. Total lipid fell from 1960 (± 172) μg per digestive gland on Day 0 (21% dry weight) to 385 (± 81) μg lipid per digestive gland on Day 19 (9% dry weight) (Table 3.1). The amount of total lipid per digestive gland differed significantly between the Day 0 samples and other days. There were no significant differences in the amount of lipid per digestive gland between all other adjacent days (e.g. between Day 3 and 6, Day 6 and 12 etc).

The lipid class composition showed significant differences between the Day 0 and starved animals in the relative levels (ie. as a proportion of total lipid) of both the free fatty acids and polar lipids (Table 3.1). Free fatty acids increased with starvation from 5 to 30% and polar lipids decreased from 77 to 46% (Table 3.1). In terms of the absolute amount of lipid class per digestive gland, polar lipids decreased from 1510 (± 225) to 177 (± 46) μg with significant differences found between the Day 0 and the starved animals on every day analysed ($p < 0.01$). Significant differences ($p < 0.01$) between all starved and the Day 0 animals were also noted in the absolute level of sterols which fell from 74 (± 2) to 19 (± 3) μg per digestive gland. There were no significant differences in levels of triacylglycerol in the digestive gland between the Day 0 and the starved animals (Figure 3.1).

3.4.2 Fatty Acids

The absolute amount of the total fatty acids per digestive gland decreased during starvation in two major episodes. By Day 3 the absolute amount of all major fatty acids had dropped to levels which remained stable until Day 12 when a further decrease was noted. All major fatty acids were subsequently maintained at these reduced levels until Day 19 of starvation (Figure 3.2).

Table 3.1 Total lipid, lipid class and lipid class ratios of the digestive gland (DG) of *Euphausia superba* during 19 day starvation. Data are the mean of three analyses \pm SE, * ANOVA comparing each day with day 0 ($p < 0.01$).

Day	0	3	6	9	12	15	19
Total Lipid (% dry weight of DG)	21.0 \pm 1.0	14.5 \pm 3.5	21.6 \pm 3.0	17.5 \pm 3.5	14.0 \pm 0.7	14.6 \pm 4.5	9.0 \pm 2.2 *
<u>Lipid Class (% of total lipid)</u>							
Triacylglycerol	16.0 \pm 4.0	15.5 \pm 6.6	16.5 \pm 0.5	18.5 \pm 6.5	19.0 \pm 5.0	25.0 \pm 7.0	20.5 \pm 2.5
Free Fatty Acid	4.5 \pm 0.5	14.0 \pm 4.0 *	20.5 \pm 8.5 *	22.5 \pm 4.6 *	30.0 \pm 0.3 *	24.5 \pm 5.0 *	29.5 \pm 0.5 *
Polar Lipid	76.5 \pm 4.5	67.0 \pm 12	59.0 \pm 8.0	54.0 \pm 2.0	45.5 \pm 4.5 *	46.5 \pm 11 *	45.5 \pm 2.5 *
Sterol	3.5 \pm 0.5	3.5 \pm 1.5	5.0 \pm 0.0	4.5 \pm 0.5	5.0 \pm 0.0	4.5 \pm 0.5	5.0 \pm 0.0
Triacylglycerol/Polar Lipid	0.2	0.2	0.3	0.3	0.4	0.5	0.5
Free Fatty Acid/Polar Lipid	0.1	0.2	0.3	0.4	0.7	0.5	0.6

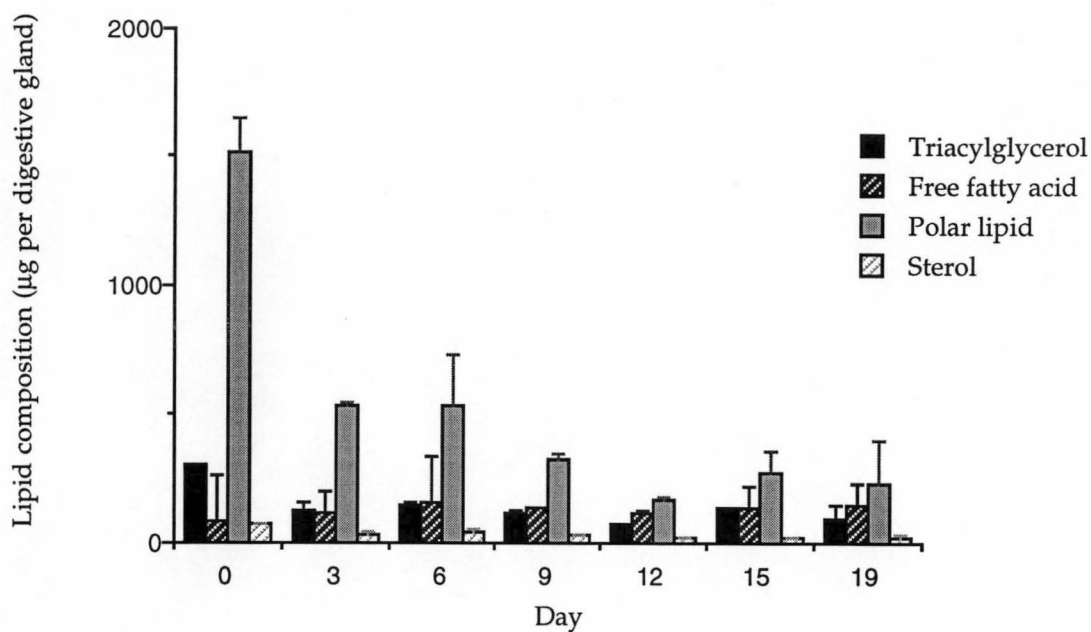


Figure 3.1 Lipid composition of total lipid in the digestive gland of *Euphausia superba* during 19 days starvation. Error bars = SE.

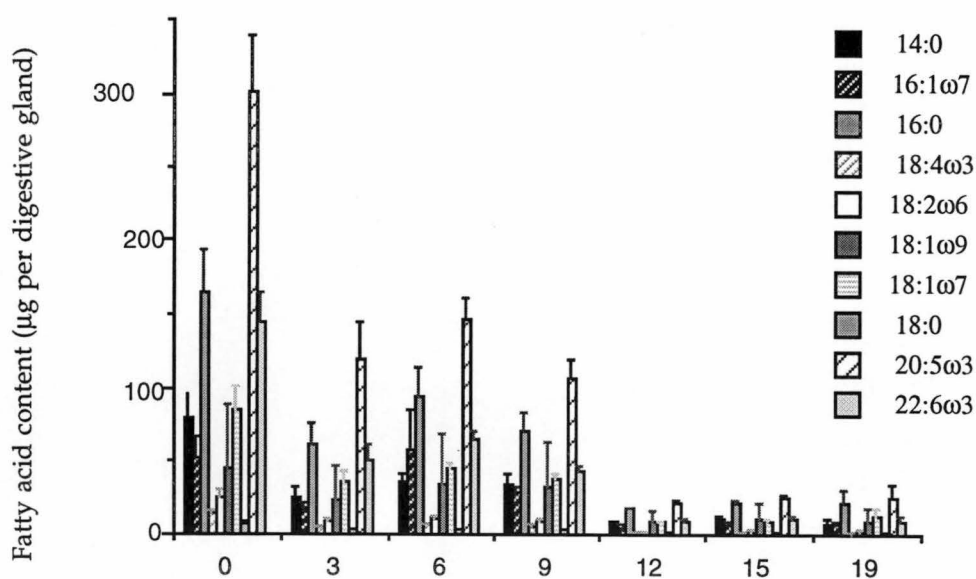


Figure 3.2 Fatty acid composition of total lipid in the digestive gland of *Euphausia superba* during 19 days starvation. Error bars = SE.

The long-chained polyunsaturated fatty acids; eicosapentaenoic (EPA) and docosahexaenoic (DHA) fell from 301 (± 38) to 26 (± 26) μg and 145 (± 21) to 10 (± 3) μg /digestive gland respectively. The total $\omega 3$ fatty acids in relative terms (ie as a proportion of total lipid) decreased significantly (using least- squares regression analysis; $p < 0.01$) from 50 to 38% over the starvation period. Although absolute levels of the total $\omega 7$ fatty acids decreased, in relative terms a significant increase was noted (from 14 to 18%, $p < 0.01$) (Tables 3.2 and 3.3). Absolute levels of 18:1 $\omega 9\text{c}$ decreased from 45 (± 32) to 9 (± 3) μg per digestive gland while relative levels increased significantly ($p < 0.01$) from 4 to 9%. The C_{16} saturated fatty acid (16:0) fell throughout starvation from 164 (± 30) to 22 (± 9) μg per digestive gland however, relative levels increased significantly ($p < 0.01$) from 16 to 21% (Table 3.2 and 3.3). A positive correlation was found between EPA and DHA using a correlation matrix (Table 3.4). This positive correlation was particularly evident between EPA and all $\text{C}_{20}\omega 3$ fatty acids. A negative correlation was noted between EPA and 16:0 and between DHA with 16:0. Negative correlations were also evident between these two PUFAs and 18:1 $\omega 9\text{c}$ (Table 3.4).

3.4.3 Sterols

Total sterol content in the digestive gland ranged from 0.7- 2% dry weight corresponding to 2- 5.5% of the total lipid. Sterols in the digestive gland in order of elution included: cholest-5-en-3 β -ol (cholesterol), cholesta-5,22E-dien-3 β -ol (22-dehydrocholesterol), cholesta-5,24-dien-3 β -ol (desmosterol), 24-methylcholesta-5,22E-dien-3 β -ol (brassicasterol), 24-methylcholesta-5,24(28)-dien-3 β -ol (24-methylene cholesterol), 24-methylcholest-5-en-3 β -ol (campesterol), 24-ethylcholesta-5,22E-dien-3 β -ol (stigmasterol), an unidentified sterol, 24-ethylcholest-5-en-3 β -ol (sitosterol) and 24-ethylcholesta-5,24(28)Z-dien-3 β -ol (isofucosterol) (Table 3.5). The unidentified C_{29} sterol with a relative retention time of 1.50 (cholesterol=1.00, sitosterol=1.63) was found in significant quantities in the digestive gland.

Table 3.2 Percentage fatty acid composition of total lipid in digestive gland of *Euphausia superba* during a 19 day starvation period. Data are the mean of three analyses \pm SE.

Percentage Composition							
Day	0	3	6	9	12	15	19
Fatty acid							
14:0	8.0 \pm 0.2	6.6 \pm 0.3	7.0 \pm 0.2	8.7 \pm 0.6	9.3 \pm 0.4	10.3 \pm 0.5	8.0 \pm 0.7
16:1 ω 7c	5.3 \pm 0.4	4.7 \pm 0.2	9.9 \pm 3.6	7.2 \pm 0.2	7.0 \pm 0.4	7.5 \pm 0.1	6.8 \pm 0.0
16:0	16.5 \pm 0.1	16.3 \pm 0.1	17.6 \pm 0.7	18.1 \pm 0.2	19.1 \pm 0.5	19.7 \pm 0.2	20.8 \pm 0.7
18:4 ω 3	1.5 \pm 0.2	1.2 \pm 0.2	1.3 \pm 0.0	1.3 \pm 0.2	1.4 \pm 0.1	1.3 \pm 0.2	1.0 \pm 0.3
18:2 ω 6	2.6 \pm 0.0	2.6 \pm 0.2	2.2 \pm 0.1	2.4 \pm 0.1	2.4 \pm 0.1	2.4 \pm 0.0	2.4 \pm 0.1
18:3 ω 3	0.7 \pm 0.1	0.6 \pm 0.0	0.7 \pm 0.1	0.7 \pm 0.0	0.7 \pm 0.0	0.7 \pm 0.0	0.8 \pm 0.0
18:1 ω 9c	3.8 \pm 2.6	6.1 \pm 0.5	6.6 \pm 0.3	7.9 \pm 1.0	9.2 \pm 0.3	9.9 \pm 0.1	9.0 \pm 0.0
18:1 ω 7c	8.6 \pm 0.1	9.8 \pm 0.3	8.6 \pm 0.6	9.6 \pm 0.5	9.6 \pm 0.2	9.1 \pm 0.3	11.2 \pm 1.2
18:0	0.8 \pm 0.0	0.8 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.0	1.4 \pm 0.3	1.1 \pm 0.0	1.1 \pm 0.1
20:5 ω 3	30.8 \pm 1.8	32.3 \pm 0.7	28.3 \pm 2.1	27.3 \pm 1.0	24.0 \pm 1.4	23.2 \pm 0.8	24.7 \pm 0.2
20:4 ω 3	1.0 \pm 0.0	1.2 \pm 0.1	0.9 \pm 0.1	0.9 \pm 0.1	0.8 \pm 0.0	0.8 \pm 0.0	0.9 \pm 0.0
20:1	0.9 \pm 0.1	0.5 \pm 0.1	0.5 \pm 0.0	0.5 \pm 0.1	0.7 \pm 0.1	0.5 \pm 0.1	0.4 \pm 0.2
22:5 ω 3	0.8 \pm 0.0	0.8 \pm 0.0	0.5 \pm 0.1	0.5 \pm 0.1	0.4 \pm 0.0	0.4 \pm 0.0	0.5 \pm 0.0
22:6 ω 3	14.7 \pm 0.6	13.6 \pm 0.0	12.4 \pm 0.8	11.1 \pm 0.6	10.2 \pm 1.4	10.0 \pm 0.1	10.0 \pm 1.0
other	4.0	3.0	2.8	3.0	3.8	2.9	2.5
16:1 ω 7c/16:0	0.3	0.3	0.6	0.4	0.4	0.4	0.3
18:1 ω 7c/18:1 ω 9c	2.3	1.6	1.3	1.2	1.0	0.9	1.2
total ω 7c	13.9	14.5	18.5	16.8	16.6	16.6	17.9
total ω 3	49.6	49.6	44.1	41.7	37.6	36.5	37.9

"Other" includes categories less than 0.5% of total fatty acids in krill analysed.

Table 3.3 Analysis of percentage fatty acid data of total lipid of digestive gland ($n=21$) of *Euphausia superba* during starvation, showing dependent variables: independent variable was "day". All data treated by least squares regression.

Fatty acid	Intercept	Regression coefficient	r ²	F	p
14:0	6.914	0.250	0.309	4.0	<0.0358
16:1 ω 7c	5.031	0.508	0.202	2.3	<0.1307
16:0	16.247	0.181	0.897	78.7	<0.0001
18:4 ω 3	1.362	0.004	0.125	1.3	<0.2993
18:2 ω 6	2.597	0.045	0.289	3.7	<0.0466
18:3 ω 3	0.634	0.006	0.520	9.8	<0.0013
18:1 ω 9c	3.802	0.673	0.745	26.3	<0.0001
18:1 ω 7c	9.071	-0.049	0.363	5.1	<0.0172
18:0	0.737	0.031	0.272	3.4	<0.0576
20:5 ω 3	32.384	-0.813	0.720	23.2	<0.0001
20:4 ω 3	1.127	-0.036	0.522	9.8	<0.0013
20:1	0.727	-0.030	0.214	2.5	<0.1149
22:5 ω 3	0.892	-0.065	0.887	70.4	<0.0001
22:6 ω 3	14.934	-0.567	0.826	42.7	<0.0001
Total ω 7c	13.072	0.449	0.505	10.2	<0.0001
Total ω 3	51.432	-1.474	0.799	35.8	<0.0001

Table 3.4 Correlation matrix using percentage composition of individual fatty acids of the digestive gland of *Euphausia superba* during a 19 day starvation period ($\bar{n}=7$). * Denotes correlations of significance interest which are discussed in the text.

	14:0	16:1 ω 7c	16:0	18:4 ω 3	18:2 ω 6	18:3 ω 3	18:1 ω 9c	18:1 ω 7c	18:0	20:5 ω 3	20:4 ω 3	20:1	22:5 ω 3	22:6 ω 3
14:0														
16:1 ω 7c	0.14													
16:0	0.59	0.35												
18:4 ω 3	0.24	-0.04	-0.53											
18:2 ω 6	-0.13	-0.98*	-0.45	0.18										
18:3 ω 3	0.31	0.36	0.78	-0.37	-0.42									
18:1 ω 9c	0.66	0.37	0.86	-0.44	-0.47	0.39								
18:1 ω 7c	-0.03	-0.26	0.61	-0.84	0.08	0.45	0.48							
18:0	0.6	-0.05	0.66	-0.06	-0.04	0.33	0.66	0.38						
20:5 ω 3	-0.8	-0.46	-0.92*	0.2	0.53	-0.63	-0.9*	-0.3	-0.73					
20:4 ω 3	-0.78	-0.64	-0.73	-0.13	0.65	-0.63	-0.68	0.04	-0.54	0.9*				
20:1	0.09	-0.36	-0.48	0.85	0.47	-0.17	-0.59	-0.56	0.07	0.29	0.04			
22:5 ω 3	-0.67	-0.7	-0.79	0.16	0.76	-0.5	-0.88	-0.16	-0.54	0.92*	0.9*	0.41		
22:6 ω 3	-0.66	-0.42	-0.92*	0.43	0.53	-0.55	-0.98*	-0.51	-0.65	0.94*	0.76	0.55	0.91*	

Table 3.5 Percentage sterol in the digestive gland of *Euphausia superba* during a 19 day starvation period. Data are the mean of three analyses \pm SE.

* ANOVA comparing each day with day 0 ($p < 0.01$).

		Percentage Composition						
Day		0	3	6	9	12	15	19
Sterol	Trivial name							
Cholesta-5,22E-dien-3 β -ol	22 -Dehydrocholesterol	6.5 \pm 1.1	2.3 \pm 0.2 *	1.6 \pm 0.2 *	1.0 \pm 0.0 *	0.7 \pm 0.1 *	0.6 \pm 0.0 *	0.7 \pm 0.2 *
Cholest-5-en-3 β -ol	Cholesterol	59.3 \pm 2.9	69.8 \pm 3.2	78.5 \pm 1.0 *	75.5 \pm 0.7 *	80.3 \pm 0.7 *	84.4 \pm 0.7 *	85.9 \pm 3.2 *
Cholesta-5,24-dien-3 β -ol	Desmosterol	8.2 \pm 2.6	17.3 \pm 1.4	16.5 \pm 0.9	20.3 \pm 0.1 *	17.6 \pm 0.8	14.3 \pm 0.5	12.5 \pm 3.6
24-Methylcholesta-5,22E-dien-3 β -ol	Brassicasterol #	5.4 \pm 1.8	0.7 \pm 0.0 *	0.7 \pm 0.1 *	0.5 \pm 0.0 *	0.2 \pm 0.0 *	0.1 \pm 0.0 *	0.1 \pm 0.0 *
24-Methylcholesta-5,24(28)-dien-3 β -ol	24 -Methylene cholesterol	1.5 \pm 0.3	0.6 \pm 0.0 *	0.1 \pm 0.1 *	0.3 \pm 0.0 *	0.1 \pm 0.0 *	0.1 \pm 0.0 *	0.1 \pm 0.0 *
24-Methylcholest-5-en-3 β -ol	Campesterol	1.3 \pm 0.5	0.3 \pm 0.0	0.2 \pm 0.1	0.2 \pm 0.0	0.2 \pm 0.0	0.1 \pm 0.0 *	0.1 \pm 0.1 *
24-Ethylcholesta-5,22E-dien-3 β -ol	Stigmasterol #	2.1 \pm 0.6	0.7 \pm 0.5	0.1 \pm 0.0 *	0.1 \pm 0.1 *	0.0 \pm 0.0 *	0.1 \pm 0.1 *	0.1 \pm 0.0 *
Unidentified sterol	-	8.2 \pm 0.7	3.5 \pm 0.6 *	1.5 \pm 0.2 *	1.6 \pm 0.3 *	0.3 \pm 0.1 *	0.1 \pm 0.1	0.1 \pm 0.1
24-Ethylcholest-5-en-3 β -ol	Sitosterol	4.8 \pm 1.4	2.8 \pm 1.8	0.5 \pm 0.0	0.5 \pm 0.4	0.5 \pm 0.2	0.1 \pm 0.0	0.5 \pm 0.0
24-Ethylcholesta-5,24(28)Z-dien-3 β -ol	(Iso) fucosterol	2.7 \pm 0.6	1.9 \pm 1.4	0.3 \pm 0.2	0.1 \pm 0.1	0.2 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0

C24 stereochemistry not determined.

The absolute levels of total sterols rose from 28 (± 6) μg on Day 0 to 62 (± 17) μg per digestive gland on Day 3 and then decreased to 5 (± 3) μg per digestive gland at the end of the starvation period. Cholesterol and desmosterol, the main sterols detected, also followed this pattern while all minor sterols ($<10\%$ of the total sterols) had fallen to near zero levels by Day 6 (Figure 3.3). The relative level of individual sterols in the digestive gland changed significantly with starvation. The unidentified C_{29} sterol fell from 8 to 0% during starvation (Figure 3.3, Table 3.5). Cholesterol increased from 59% (± 3) on Day 0 to 85% on Day 19 (± 0.5). The incremental increase in cholesterol was significant ($p < 0.01$) on each day sampled throughout starvation compared with the digestive gland of the Day 0 krill. Desmosterol increased from 8 (± 3) to 20 (± 0.1)% until Day 12, and then fell to 12 (± 4)% by Day 19. There was no significant difference however, in the absolute levels of cholesterol and desmosterol throughout starvation.

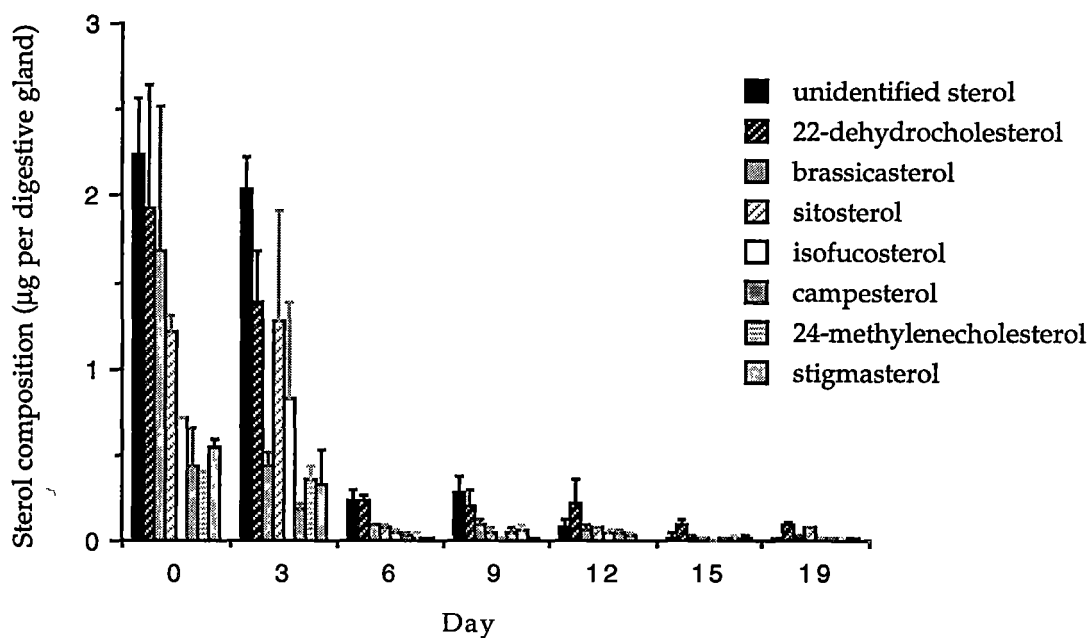


Figure 3.3 Sterol composition of total lipid in the digestive gland of *Euphausia superba* during 19 days starvation (cholesterol and desmosterol not included). Error bars = SE.

3.5 Discussion

The digestive gland in crustaceans is thought to play a major role in lipid metabolism. This organ is generally regarded as having several functions, including digestion, absorption and short-term storage of lipids from the gut (Dall et al. 1992). In the present study, the lipids in the digestive gland of *Euphausia superba* appeared to be utilised as an energy source throughout short-term starvation, as evidenced by the substantial decrease in total lipid. Major changes occurred in the relative levels of individual lipid classes in the digestive gland. Polar lipid was the major constituent, which may be a reflection of the lipid constituents of the algal diet of *Euphausia superba*. Volkman et al. (1989) reported that polar lipid was the dominant lipid class for microalgal species commonly used in mariculture harvested during logarithmic growth phase, with levels ranging from 44 to as high as 99% of the total lipid. Similarly, polar lipid was the dominant lipid class in both logarithmic and stationary phase harvested *Phaeocystis pouchetii*, a major southern ocean algal species (Nichols et al. 1991). Polar lipids of the digestive gland decreased substantially during starvation in this study. By Day 12, polar lipid levels were equivalent to those found in the whole body of *Euphausia superba* which are approximately 40-50% of the total lipid (Chapter 4, Virtue et al. 1993a).

Triacylglycerols in contrast, although decreasing marginally throughout starvation, increased in proportion to the decreasing weight of the digestive gland. Triacylglycerols have been reported as being a form of overwintering lipid in *Euphausia superba* (Hagen 1988), however, in this study triacylglycerols in the digestive gland were not utilised in significant quantities compared to polar lipids during short-term starvation. The triacylglycerol/polar lipid ratio increased during starvation from 0.2, indicative of a typical algal ratio, to 0.5 which is only half that of the ratio for whole *Euphausia superba* (approximately 1.0) where both triacylglycerol and polar lipid generally are maintained at similar levels (Virtue et al. 1993a). The triacylglycerol/polar lipid ratio does not differ significantly in whole animals during starvation of up to 30

days (unpublished data) which would imply that both lipid classes are metabolised at similar rates.

Relative levels of free fatty acids in the digestive gland increased during starvation. A rise in unesterified fatty acids during starvation is usually a result of the lipolysis of triacylglycerol which is a major energy yielding process (Stryer 1988). In the digestive gland of *Euphausia superba*, however, it would appear that polar lipids are being metabolised as a source of metabolic energy, contributing to the endogenous pool of free fatty acids, which increases with starvation.

During starvation, shrinkage has been reported in *Euphausia superba* which results from body tissue break down. Ikeda and Dixon (1982) reported shrinkage was the mechanism responsible for krill surviving up to 211 days without food in the laboratory. They determined, using C:N ratios, that body protein rather than lipid was being utilised as the major energy source during starvation. Breakdown of body tissue would presumably result in the release of a substantial amount of polar lipid from cell membranes. As polyunsaturated fatty acids (PUFA) are major constituents of the polar lipid fraction in *Euphausia superba* (Chapter 4, Virtue et al. 1993a), catabolism of polar lipid during shrinkage would entail a concomitant release of PUFA. In this study polar lipid in the digestive gland remained the dominant lipid class, although absolute levels decreased with starvation. The polar lipids of body cells, essentially the phospholipid and glycolipid of the lipid bilayer of cell membranes, may be mobilised and transported to the digestive gland either for redistribution or as a source of metabolic energy during starvation, however this is impossible to validate further without the use of labelled substrates. Alternately, changes in the levels of polar lipids may simply reflect stored dietary lipids being utilised during short-term starvation. Nicol et al. (1992) reported shrinkage of *Euphausia superba* occurred following 10-20 days of captivity with a reduced food supply. The duration of the starvation period in this study may not have been long enough to reflect biochemical changes in the lipid class constituents occurring during shrinkage.

Although the lipid class profile resembled that expected in dietary phytoplanktonic species, the fatty acid profile in the digestive gland does not appear to be solely the result of absorption and storage of dietary fatty acids. The total $\omega 3$ fatty acids in the digestive gland were initially as high as 50% of the total fatty acids decreasing to 38% during starvation. Of this initial 50%, up to 45% was contributed by the essential fatty acids, EPA and DHA, which decreased to 35% during starvation. Dall et al. (1992) showed that a large part of the newly-assimilated fatty acids are incorporated into the polar lipid fraction of the digestive gland of the tiger prawn, *Penaeus esculentus*, for transport to other tissues.

Levels of EPA and DHA in algal species on which krill are reported to graze are substantially lower than those found in the digestive gland in this study and hence newly-assimilated fatty acids cannot account for these high PUFAs levels. Nichols et al. (1986) for example reported levels of both EPA and DHA to be around 10% in the Antarctic sea ice diatom *Nitzschia cylindrus* and levels reach 20% in sea-ice diatom communities (Nichols et al. 1993). The abundant Antarctic prymnesiophyte *Phaeocystis pouchetii* has quite low levels (3-4%) of EPA and DHA (Nichols et al. 1991, Virtue et al. 1993a).

A possible carnivorous dietary input may be responsible for high PUFA levels in the digestive gland or this fatty acid profile may simply be indicative of the biochemical capabilities of *Euphausia superba*. Chain elongation and desaturation of dietary fatty acids could be occurring in this organ, with the amount of conversion decreasing with increasing starvation. The conversion of exogenous shorter chain fatty acids to PUFA has been suggested in *Euphausia superba* fed a diet deficient in 20:5 $\omega 3$ (Chapter 4, Virtue et al. 1993a). Dall et al. (1992) suggested that the catabolism of cell membranes during starvation was a sufficient source of 20:5 $\omega 3$ and 22:6 $\omega 3$ and that these fatty acids may not be essential in the tiger prawn *Penaeus esculentus*.

The fatty acid dynamics in the digestive gland were highlighted using a statistical correlation matrix (Table 3.4). Relative levels of both EPA and DHA decreased throughout starvation. In this organ both these fatty acids were utilised at similar rates in this organ during starvation as was evident in the positive correlation found between them ($p=0.94$). A

positive correlation was also found between EPA and other C20 ω 3 fatty acids. Although all fatty acids decreased in absolute levels over starvation, in relative terms the saturated fatty acids increased. Preferential utilisation of PUFAs compared to saturated fatty acids throughout starvation was evident in the negative correlation found between EPA and 16:0 ($p = -0.92$) and between DHA and 16:0 ($p = -0.92$). Negative correlations were also found between PUFAs and both 14:0 and 16:0, however not to the same extent. Monounsaturated fatty acids had a similar profile to saturated fatty acids. Negative correlations were found between PUFAs and mono unsaturated fatty acids in particular 18:1 ω 9c.

Cholesterol was the major sterol found in the digestive gland of *Euphausia superba* and no significant differences were detected in absolute levels of cholesterol and desmosterol throughout starvation. Cholesterol is assumed to be an important constituent of cell membranes and subcellular structures in crustaceans (Teshima 1982). Crustaceans do not have the capacity for *de novo* sterol synthesis but are able to dealkylate dietary C₂₈ and C₂₉ sterols to cholesterol. Using radioisotope labelled sterols, crustaceans have been reported to convert 24-methyl cholesterol and brassicasterol to cholesterol via a desmosterol intermediate (Teshima 1982).

The majority of sterols found in the digestive gland of *Euphausia superba* probably reflect that of their diet with some *in vivo* sterol inter conversion. The digestive gland may be the site of sterol conversion, where dietary sterols are dealkylated to cholesterol and desmosterol. In this study absolute levels of minor sterols of algal origin were substantially depleted by Day 6 of starvation. The rate of depletion of sterols of algal origin in the digestive gland may be indicative of the rate of sterol conversion in *Euphausia superba*.

Just as algal pigments, such as xanthophyll, can be used to indicate phytoplankton composition in seawater samples, analysis of the sterol as well as photosynthetic pigment composition in the digestive gland has the potential to provide relevant information regarding recent feeding activity of *Euphausia superba*. Chlorophyll degradation products have been used to measure grazing rates of planktonic herbivores (Nemoto 1986, Shuman and Lorenzen 1975). Unlike chlorophyll which is degraded in the acidic conditions of the stomach (Shuman and Lorenzen 1975), sterols appear to

maintain integrity in the digestive gland of *Euphausia superba* prior to any conversion to cholesterol and its intermediates. There are some apparent differences in the sterol content and composition in various species and classes of phytoplankton. Dinosterol and dinostanol for example are used as biological markers for dinoflagellates (Nichols et al. 1984). Dinosterol was not detected in the digestive gland of *Euphausia superba* in this study which would indicate dinoflagellates were absent from the recent diet of these animals. Algal sterols in the gut contents of zooplankton are potential indicators of dietary composition. Pigments also, such as peridinin which is the dominant carotenoid in dinoflagellates (Strain et al. 1971) and fucoxanthin like pigments which are the major components in prymnesiophytes (Wright and Jeffrey 1987), can be used together with sterols in the digestive system of marine herbivores to examine diet composition.

Algal sterols are not only useful indicators of dietary composition but they potentially can be used for the quantitative determination of various phytoplanktonic components in the diet of zooplankton. If the amount of a particular sterol in an algal species is known, the approximate input of this species in the diet can be determined. In the prymnesiophyte *Phaeocystis pouchetii* for example, 0.24 pg of sterol was reported per algal cell of which up to 100% was brassicasterol (Nichols et al. 1991). In the digestive gland of *Euphausia superba* in this study, 1.6µg of brassicasterol was detected on Day 0 which, if originating solely from *Phaeocystis pouchetii* would have involved the ingestion of at least 6.6×10^5 cells. However, the situation is far more complicated than this as the diet of *Euphausia superba* can often be of a mixed planktonic nature. Many algal species may have similar sterol profiles and information on both the sterol composition and the absolute amounts of sterols in key algal species that comprise the diet of *Euphausia superba* diet has yet to be determined. The use of sterols in conjunction with pigments, however, is potentially a viable alternative to the tedious microscopic methods used to determine algal dietary content and composition. Non-thecate forms of algae are not accounted for by microscopy and therefore estimations of gut algal composition using this method are limited. Techniques using both sterols and pigments in the analysis of dietary composition of zooplankton warrant further development.

In summary the digestive gland of *Euphausia superba* serves in the absorption and short-term storage of lipids from the gut. In terms of lipid dynamics, the digestive gland plays a major role during short-term starvation. Polar lipid, the main lipid class, and EPA, DHA and 18:0, the major fatty acids, were depleted within 9 days of starvation. Most dietary derived sterols were depleted within 6 days of starvation. Levels of both free fatty acids and polar lipids in the digestive gland may be used as an index of feeding history of *Euphausia superba* in the field. Certain sterols in the digestive gland of *Euphausia superba* may be used as biomarkers to detail recent dietary content and composition.

EPA and DHA levels in the digestive gland of *Euphausia superba* are higher than that able to be obtained from a phytoplankton diet and hence are obtained from either elongation and desaturation of shorter chained dietary fatty acids or tissue catabolism. This finding indicates that EPA and DHA may not be essential fatty acids in *Euphausia superba* as has been proposed for the tiger prawn *Penaeus esculentus* (Dall et al. 1992).

Addendum

The research in this paper was carried out by myself. Co-authors Peter Nichols and Stephen Nicol played a supervisory role. Both co-authors were my PhD research supervisors.

Chapter 4. The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim.

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4.1 Abstract

The fatty acid profiles of *Euphausia superba*, the Antarctic prymnesiophyte, *Phaeocystis pouchetii*, and a temperate diatom, *Phaeodactylum tricornutum* were analysed and compared. The lipid content, lipid class, fatty acid and sterol composition of *Euphausia superba* fed diets of these cultured phytoplankton and a mixed diet of both phytoplankton species, were determined. No significant difference was found between total lipid levels of *Euphausia superba* reared on each of these different diets. *Phaeocystis pouchetii*, although being deficient in a number of what are normally referred to as the essential fatty acids, is an adequate food source for *Euphausia superba*. The proportion of polyenoic fatty acids varied within lipid classes although there was no significant difference between levels of the long chain polyunsaturate 20:5 ω 3 in the total lipid of *Euphausia superba* fed on these diets. This acid was found to be less than 1% of the total lipid in *Phaeocystis pouchetii* compared to 37% in *Phaeodactylum tricornutum*. This result indicates that krill may possess the ability to convert exogenous shorter chain fatty acids to the long chain polyunsaturates, 20:5 ω 3 and 22:6 ω 3. Significant differences were detected between krill fed the diatom compared to the prymnesiophyte diet in the ratio of 16:1 ω 7c/16:0. Significant differences were also detected in several shorter chain fatty acids and between fatty acids within their lipid classes. Such differences may have the potential to be used as biochemical signatures in terms of providing information on food sources and possible feeding grounds of *Euphausia superba*. *Phaeocystis pouchetii* in a very late stationary phase, although not used in this feeding study, was found to contain 11% 22:6 ω 3. This fatty acid was found between 3-4% in cultures analysed during exponential growth phase. Only a slight increase, however, was detected in levels of 20:5 ω 3 between growth phases. Further investigation into the fate of the late stationary growth phase of *Phaeocystis pouchetii* in the field are required as there are few substantive sources of 22:6 ω 3 in natural algal populations.

4.2 Introduction

Euphausia superba (Antarctic krill) plays a crucial role in the Southern Ocean ecosystem as most of the vertebrate populations depend on it either directly or indirectly for their survival. This pelagic euphausiid is of considerable commercial importance. A 357,538 tonne harvest in 1990/91 (Anon. 1991) makes the krill fishery the largest single species crustacean fishery in the world (Nicol 1990). The biochemical composition of *Euphausia superba* has been the subject of numerous investigations (e.g. Clarke 1980, Clarke 1984, Fricke et al. 1984). Unlike other Antarctic euphausiids and copepods, which store large amounts of wax esters accumulated during the summer and spring, there is little substantive evidence that *Euphausia superba* synthesises a substantial overwintering lipid store (Bottino 1974, Clarke 1980, Hagen 1988). Krill probably possess the ability to feed on a variety of food sources (Clarke 1980) including *Phaeocystis pouchetii* an abundant cosmopolitan prymnesiophyte which is reported as being a major algal component within the sea ice (Garrison et al. 1987, Garrison and Buck 1989), at the ice edge and in the open ocean during the austral winter (Ashworth et al. 1990, Davidson and Marchant, 1992a). *Phaeocystis pouchetii* is frequently the first species to bloom in polar waters (Lutter et al. 1989, Davidson and Marchant 1992b) at what would constitute a critical period when the nutritional status of most species, in particular the herbivores, is lowest.

Phaeocystis pouchetii appears to be a significant carbon source in the Southern Ocean (Fryxell and Kendrick 1988, Davidson and Marchant 1991, Davidson and Marchant, 1992a), but has been reported as having insufficient nutritional quality to support growth and reproduction of grazers (Al-Hasan et al. 1990, Claustre et al. 1990). Biochemically this species appears to be a poor food source, being low in vitamin C and the essential fatty acids; eicosapentaenoic (20:5 ω 3) and docosahexaenoic (22:6 ω 3) (Claustre et al. 1990, Nichols et al. 1991, Davidson and Marchant, 1992a). Studies in Norwegian and German waters, however, have reported copepods actively grazing *Phaeocystis pouchetii* (Weisse 1983, Tande and Båmstedt 1987). In a related study, Sargent et al. (1985) found an algal slick, during the end of a bloom of *Phaeocystis pouchetii*, to

contain high levels of the essential fatty acids, which may be a major source of polyunsaturated fatty acids (PUFA) to these zooplankton in arctic-boreal waters. Feeding studies and gut content investigations have suggested that some species, including *Euphausia superba*, are unable to digest *Phaeocystis pouchetii* (Sieburth 1960, Marr 1962, Pieters et al. 1980, Verity and Smayda 1989) while other species overtly avoid its blooms (Rogers and Lockwood 1990). Apart from evidence revealing scales and thread-like material produced by *Phaeocystis pouchetii* in the gut and faecal material of wild krill (Marchant and Nash 1986), the relationship between these two major Southern Ocean species remains a conundrum.

Algae, being autotrophic organisms, must synthesise all their own fatty acids. Phytoplankton species with high levels of polyunsaturated fatty acids, such as diatoms, have traditionally been characterised as a food source of high nutritional value (eg. Brown et al. 1989, Claustre et al. 1988/89). Polyunsaturated fatty acids clearly have a vital nutritional function for grazing zooplankton as they play a major physiological role. Crustaceans have been widely reported as being incapable of synthesizing polyunsaturated fatty acids *de novo* (Clarke 1983) however, the varying ability to biosynthesise fatty acids is relatively species specific. Dietary sources of the polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic are considered essential in many crustacean species (Kanazawa 1984).

The aim of this study is to elucidate, in terms of lipid composition data, the relationship between *Euphausia superba* and *Phaeocystis pouchetii*. It is also designed to provide an initial examination on the question of whether *Euphausia superba* possesses the biosynthetic mechanisms necessary to satisfy their need for long chain polyunsaturated fatty acids or whether they merely bioaccumulate their dietary fatty acids. The diatom *Phaeodactylum tricornutum*, is used as a comparative food source to *Phaeocystis pouchetii* in this study for several reasons. The two algae are markedly different in their levels of eicosapentaenoic acid yet equally deficient in docosahexaenoic acid. The difference in levels of eicosapentaenoic acid and this deficiency in docosahexaenoic acid allows the determination of krill's capacity to biosynthesis 20:5 ω 3 and to elongate and further desaturate 20:5 ω 3 to 22:6 ω 3. Although not being an Antarctic species, *Phaeodactylum tricornutum*, has routinely been used

to maintain krill at the Australian Antarctic Division for many years due to its prolific growth rate. It has been considered an adequate food source for krill and considerable biological information is available from these culture conditions (Ikeda and Dixon 1982, Ikeda and Thomas 1987, Nicol and Stolp 1989).

Krill fed a mixed diet of these two phytoplankton were also investigated to compare their lipid composition to that of the krill fed single species diets. A review by Brown et al. (1989) suggests increased growth rates of zooplankton fed more than one algal species is attributed to complementary nutrients and micronutrients, and differences in algal digestibility. In addition to the nutritional aspects of a mixed algal diet, the extent to which potential biochemical marker compounds would be manifested in krill fed a mixed phytoplankton diet containing diatoms and prymnesiophytes was investigated. For the identification of biomarkers, consideration must be given to the observation that krill have been reported to eat diatoms, choanoflagellates and other phytoplankton (Miller and Hampton 1989).

Various lipids may have a dietary origin in an organism and these lipids can be used as biological markers to probe trophic relationships (Mayzaud et al. 1976, Sargent and Whittle 1981, Claustre et al. 1988/89). The extent to which the fatty acid and sterol composition reflects that of the diet may be dependant on the biosynthetic capabilities of the animal, together with other complex physiological factors, which can be markedly species specific. The development of such biological signatures in *Euphausia superba* may help address fundamental ecological parameters such as their feeding grounds and food sources. In this study the lipid class, fatty acid and sterol composition of *Euphausia superba* fed on various controlled algal diets are compared. The information obtained is used to examine the possible incorporation of dietary lipid by *Euphausia superba* under controlled conditions. These results will, in the future, be used for comparison with field data.

4.3 Material and Methods

Specimens of *Euphausia superba* were caught in January 1989 in the Prydz Bay region of Antarctica and were subsequently maintained in the laboratory cold room of the Australian Antarctic Division, Kingston, Tasmania prior to the experimentation period. Krill were maintained in polyethylene buckets at 0°C in darkness. The water was changed weekly and animals were fed with an algal culture of *Phaeodactylum tricornutum* (10^5 - 10^6 cells ml⁻¹).

The three different diets were investigated using fifteen sexually immature krill of similar size and weight. Five krill were incubated at each of the three following feeding regimes: *Phaeocystis pouchetii* (10^5 - 10^6 cells ml⁻¹ week⁻¹), *Phaeodactylum tricornutum* (10^5 - 10^6 cells ml⁻¹ week⁻¹), and an equal mixture of these two phytoplankton.

Krill were incubated separately in 2L containers and starved for 10 days prior to the five month feeding period. At the end of this period krill were rinsed in Milli-Q® water and quick frozen to -40°C for a maximum of 30 minutes prior to extraction. Triplicate analysis of krill at each of the feeding regimes were performed. Krill triplicates incorporated two batches of two and a single individual. All wet weights of krill were converted into dry weights using a mean value of 77.2% water content which allowed for variation throughout the moult cycle (Nicol et al. 1992).

Several axenic strains of cultured *Phaeocystis pouchetii* isolated from Prydz Bay, Antarctica were used and analysed: strain DE 12.1 (a flagellate strain), DE 11 (a colonial strain) and DE 15.1 (a mixed strain of both colonial and flagellate stages) (Australian Antarctic Division Culture Collection, Hobart, Australia). Cultures of *Phaeocystis pouchetii* were grown in a 0.05 µm filtered GP5 medium (Loeblich and Smith 1968) in glass flasks at 4°C on a 12/12 hr dark/light cycle under cool white fluorescent tubes at an intensity of 6.19 ± 0.76 W m². Cultures of *Phaeodactylum tricornutum* was cultured using a 0.05 µm filtered f/2 medium (Guillard and Ryther 1962) at 10°C on a 12/12 hr dark/light cycle. Food concentrations were determined microscopically using a haemocytometer with double neubauer ruler. All healthy cells in the graticule were counted and three counts were averaged.

All strains of phytoplankton were harvested for analysis and/or fed to krill during exponential growth phase. Phytoplankton used for lipid analysis was filtered onto precombusted (450°C) Whatman GF/F. One culture of *Phaeocystis pouchetii* was allowed to reach a very late stationary phase (two months) prior to harvesting for analysis. This culture was not used in the feeding study; it was analysed for comparison of the fatty acids from the exponential and late stationary life stages. Only the mixed flagellate and colonial culture of *Phaeocystis pouchetii* (DE15.1) was used in the feeding experiment. All phytoplankton samples were extracted and analysed in duplicate. Procedures for lipid extraction and analysis of krill and phytoplankton were the same as those described in Chapter 2.

4.3.1 Statistics

The triplicate means for the total lipids, fatty acids, and sterols were determined for all krill analysed. Phytoplankton samples were analysed in duplicate and means reported. All statistical analyses were performed using a three way analysis of variance (ANOVA) using the Statistical Analysis System (SAS Version 6.03, 1988). All statistical analyses of the fatty acid and sterol profiles for the total lipids were reported. Only fatty acid comparisons of triacylglycerol between diets, polar lipid between diets and glycolipid between diets were reported (ie. fatty acid comparisons between triacylglycerol and polar lipids, triacylglycerol and glycolipid, and polar lipid and glycolipid were not relevant). The fatty acid profiles of lipid classes within diets, where a significant difference was detected, were reported. Where there were no significant differences between diets of fatty acid profiles of an individual lipid class, statistical results were not reported (Tables 4.3 and 4.4). Arcsin transformation was performed on all percentage data used for statistical analysis.

4.4 Results and Discussion

4.4.1 Krill lipid, composition and content

Krill lipid content ranged from 7.3% for the *Phaeocystis* diet to 10.2% in the *Phaeodactylum* diet (Table 4.1). There was no statistically significant differences between the lipid content of krill fed on the three experimental diets ($F_{2,6} = 0.631$, $p = 0.56$). Lipid content of the krill in this experiment, although low compared to field samples of sexually immature krill (Reinhardt and Van Vleet 1986), compared favourably to field values of mature males and post-spawning females (Everson 1976, Clarke 1980) and batched samples of krill (Saether et al. 1985). The lipid content of wild *Euphausia superba* collected between spring and fall is highly variable between sexes, maturity status and schools, ranging from 3-36% dry weight (Clarke 1980, Ellingsen and Mohr 1981, Saether et al. 1985, Hagen 1988, Nicol et al. 1992).

Table 4.1 Dry weight, lipid class dry weight and lipid class ratios of *Euphausia superba* fed on *Phaeocystis*, *Phaeodactylum* and a mixture of these two phytoplankton: all data mean of triplicate analyses \pm SE.

	<i>Phaeocystis</i>	<i>Phaeodactylum</i>	Mixed diet
Krill dry weight (g)	0.18 \pm 0.04	0.18 \pm 0.03	0.19 \pm 0.03
Total Lipid (% dry weight)	7.3 \pm 1.4	10.2 \pm 0.4	8.9 \pm 1.6
Lipid per individual krill (mg)	4.4 \pm 1.4	4.3 \pm 1.2	5.2 \pm 0.1
<u>Lipid class (mg/g dry weight)</u>			
Polar lipid	37.4 \pm 5.6	44.7 \pm 1.8	43.1 \pm 4.4
Triacylglycerol	28.6 \pm 8.4	49.7 \pm 5.4	37.8 \pm 10.6
Sterol	4.2 \pm 1.3	5.5 \pm 0.8	5.5 \pm 1.0
Free fatty acid	2.7 \pm 0.6	2.3 \pm 0.2	2.4 \pm 0.3
Triacylglycerol/polar lipid	0.7 \pm 0.2	1.1 \pm 0.2	0.7 \pm 0.2
Free fatty acid/polar lipid	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0

The lipid composition was dominated by polar lipids (including phospholipids and glycolipids) and triacylglycerols. The distribution of the major lipid classes varied with diet although no statistically significant difference was found between amounts of the lipid classes for all diets (Figure 4.1). Sterols were in the range of 4.4-6.5% of the total lipids. Non-esterified (free) fatty acids were only minor components of the total lipids (2.9-4.4%; 2.5-3 mg/g dry weight; Table 4.1). The process of digestion releases only small quantities of free fatty acid in the gut which are then rapidly re-esterified once they transverse the gut wall (Clarke 1987). A higher percentage of free fatty acid could also indicate inadequate storage prior to extraction.

The ratio of neutral to polar lipids in the krill varied with diet. The highest ratio was found in the *Phaeodactylum* fed krill whereas krill fed the *Phaeocystis* and the mixed diet had similar ratios. No difference was found in the ratio of free fatty acid to polar lipid between diets (Table 4.1)

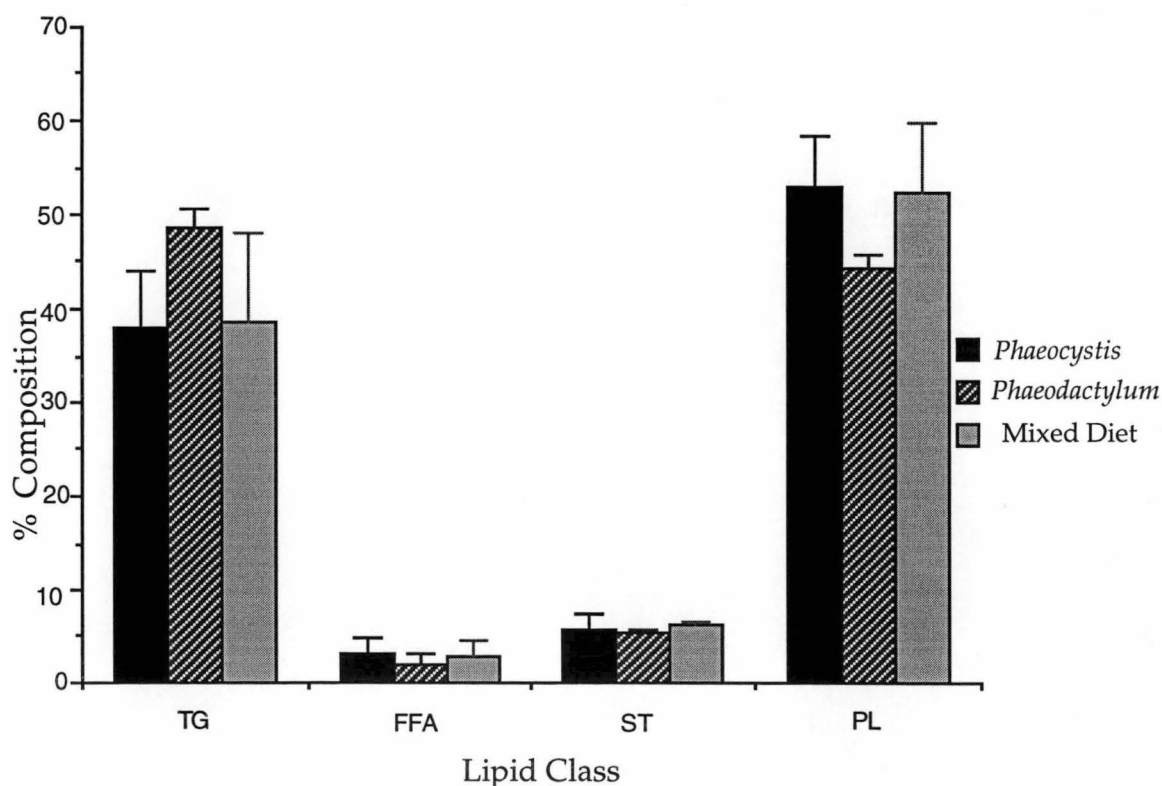


Figure 4.1 Percentage composition of lipid classes of *Euphausia superba* fed *Phaeocystis*, *Phaeodactylum* and a mixed diet of these two phytoplankton. TG = Triacylglycerol, FFA = Free Fatty Acid, ST = Sterol, PL = Phospholipid. Vertical bars = standard error.

4.4.2 Phytoplankton fatty acid composition

There are marked differences between the fatty acid composition of both algal species and between growth phases of *Phaeocystis pouchetii* (Table 4.2). All strains of *Phaeocystis pouchetii* examined, except for the culture in late stationary phase, were found to be very low in the two essential polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic, as reported for other cultured strains of this species isolated from Prydz Bay, Antarctica (Nichols et al. 1991) and field samples from the eastern Irish Sea and the Arabian Gulf (Al-Hasan et al. 1990, Claustre et al. 1990). In contrast, analysis of field slicks of *Phaeocystis pouchetii* from Norwegian waters reported high levels of the essential fatty acids (Sargent et al. 1985) which Nichols et al. (1991) suggested may be due to biochemical differences between strains, or more likely, a result of diatom influence in the sample. Analysis of a *Phaeocystis pouchetii* strain harvested in very late stationary phase during this study was found to be high in 22:6 ω 3. It would be unusual for the alga to reach an equivalent age in Antarctic waters as this source of fixed carbon is respired by microheterotrophs and bacteria (Davidson and Marchant 1992a, Davidson and Marchant, 1992b). Nevertheless this finding highlights the need for further investigations into the use of late stationary growth phase of microalgae, in particular *Phaeocystis pouchetii*, in terms of nutritional aspects for prey organisms. In addition, this finding has implications pertinent to the mariculture industry. Relatively few species of microalgae are known to contain very high levels of 22:6 ω 3 and *Phaeocystis pouchetii* may prove to be an important source of this essential fatty acid.

The diatom, *Phaeodactylum tricornutum*, was used to compare levels of eicosapentaenoic acid with that of the prymnesiophyte while simulating its deficiency in docosahexaenoic acid. *Phaeodactylum tricornutum* has proven to be an adequate food source for *Euphausia superba* in the laboratory (Ikeda and Thomas 1987). *Phaeodactylum tricornutum*, however, has been ranked as a fairly low quality food source for the mariculture industry (Brown et al. 1989) and has been found deficient in the amino acid tryptophan (Epifanio 1981). More recently, following extensive algal screening, Yongmanitchai and Ward (1991) found *Phaeodactylum tricornutum* to be high in 20:5 ω 3 (9-32%, depending on culture conditions) and low in 22:6 ω 3, which is similar to results in this study of 37% 20:5 ω 3 accompanied by 2.7% 22:6 ω 3 (Table 4.2).

Table 4.2 Fatty acid composition. *Phaeocystis* strains examined: fl = flagellate, cl = colonial, mx= mixed flagellate and colonial, st= *Phaeocystis* in very late stationary phase. All data is the mean of duplicate analyses.

Species Mixture					
Fatty acid	<i>Phaeodactylum</i>	<i>Phaeocystis</i> fl	<i>Phaeocystis</i> mx	<i>Phaeocystis</i> cl	<i>Phaeocystis</i> st
14:0	4.6	34.5	14.0	38.3	33.6
15:0	0.1	1.1	-	-	-
16:4 ω 1	10.9	-	-	-	-
16:3 ω 4	5.5	-	-	-	-
16:1 ω 7c	23.8	5.8	3.2	13.3	5.5
16:1 ω 13t	1.1	-	-	-	-
16:1 ω 5c	0.1	0.9	-	0.3	0.7
16:0	8.5	16.9	3.0	13.4	15.2
18:5 ω 3	-	2.9	60.7	12.6	-
18:4 ω 3	0.5	4.2	7.5	2.1	6.3
18:2 ω 6	2.5	3.3	1.8	4.0	2.9
18:3 ω 3	0.7	1.2	2.5	0.8	1.0
18:1 ω 9c	0.5	19.5	2.6	10.0	17.8
18:1 ω 7c	-	2.9	0.6	1.4	2.6
18:0	0.2	2.1	0.2	0.8	1.7
20:5 ω 3	36.6	0.7	0.8	-	1.9
22:6 ω 3	2.7	3.8	3.1	3.1	11.0
other	1.7	0.2	0.1	0	0

"Other" includes categories less than 0.5% fatty acid in all phytoplankton.

4.4.3 Fatty acid composition of krill lipid classes

Fatty acid composition of the total lipid and the three lipid classes examined (triacylglycerol, glycolipid and phospholipid) are shown in Table 4.3. Long chain polyunsaturated fatty acids; eicosapentaenoic and docosahexaenoic, dominated in the phospholipid fraction. Polyunsaturated fatty acids (PUFA) are likely to have been diverted preferentially to phospholipids to serve a structural function in membrane lipids.

Phaeocystis fed krill were significantly higher in 22:6 ω 3 than krill reared on the *Phaeodactylum* diet which is a direct reflection of the fatty acid profiles found in the two phytoplankton species ($F_{1,4} = 10.961$, $p = .0296$ Fisher PLSD = 3.291). There were no significant differences in the levels of 20:5 ω 3 in the total lipid from animals fed the three diets although there were significant differences found between the lipid classes in the animals fed the *Phaeocystis* and *Phaeodactylum* diets and between animals fed the *Phaeocystis* and the mixed diet (Table 4.3).

Considering total PUFA levels, there was no significant difference between diets. *Euphausia superba* was found capable of utilising *Phaeocystis pouchetii* as efficiently as *Phaeodactylum tricornutum* as a dietary source of lipids essential for structural maintenance and energy. Previous studies using zooplankton raised on algal diets deficient in PUFA found low PUFA levels in their tissue (Scott and Middleton 1979, Watanabe et al. 1983 and Walford and Lam 1987). In contrast to the evidence that levels of PUFAs in zooplankton are being influenced by their algal diet, this study indicates *Euphausia superba* maintains high relative levels of PUFAs independent of dietary PUFA composition (Table 4.3). Tanoue and Hara (1986) reported that *Euphausia superba* fecal pellets contained elevated unsaturated fatty acids compared with their phytoplankton food source. Results from feeding studies using radioactive labelled linolenic acid have shown that the capacity of marine crustaceans to elongate and desaturate long chain ω 3 PUFA is poor (Kanazawa et al. 1978).

Table 4.3 Percentage fatty acid composition of *Euphausia superba* fed on *Phaeocystis*, *Phaeodactylum* and a mixed diet of these two phytoplankton: all data are mean of triplicate analyses \pm SE. TL= Total lipid, TG=Triacylglycerol, GL= Glycolipid, PL= Phospholipid. Pc*Pd=*Phaeocystis* diet verses *Phaeodactylum* diet, Pc*Mx=*Phaeocystis* diet verses Mixed diet, Pd*Mx=*Phaeodactylum* diet verses Mixed diet. *P \leq 0.05; **P \leq 0.01; ***P \leq 0.001

ANOVA																									
Phaeocystis Diet					Phaeodactylum Diet				Mixed Diet				Total Lipid			TG		TG		GL		GL		PL	
Fatty acid	TL	TG	GL	PL	TL	TG	GL	PL	TL	TG	GL	PL	Pc*Pd	Pc*Mx	Pd*Mx	Pc*Pd	Pd*Mx	Pc*Pd	Pc*Mx	Pc*Pd	Pc*Mx	Pc*Pd	Pc*Mx	Pc*Pd	
14:0	10.2 ± 1.3	17.5 ± 0.5	16.3 ± 1.2	2.4 ± 0.2	11.4 ± 1.9	16.3 ± 1.4	15.5 ± 1.0	1.8 ± 0.6	7.7 ± 0.4	16.4 ± 1.3	14.9 ± 2.2	2.0 ± 0.1	ns	*	**	ns	ns	ns	ns	ns	*				
15:0	-	-	0.1 ± 0.1	-	-	-	0.6 ± 0.1	-	-	-	1.1 ± 0.6	-	*	ns	**	ns	ns	*	**	ns					
16:4ω1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.3	-	0.5 ± 0.1	0.9 ± 0.1	1.2 ± 0.4	-	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	-	ns	ns	ns	ns	ns	**	ns	ns					
16 PUFA	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	-	0.4 ± 0.1	1.2 ± 0.4	1.5 ± 0.4	-	0.7 ± 0.3	0.6 ± 0.1	0.5 ± 0.1	-	ns	ns	ns	ns	ns	***	*	ns					
16:1ω7c	7.3 ± 0.4	12.5 ± 0.1	12.8 ± 0.6	3.3 ± 0.5	10.9 ± 0.9	19.8 ± 3.4	18.5 ± 3.1	5.1 ± 1.0	11.4 ± 2.8	14.8 ± 0.8	14.7 ± 0.9	3.8 ± 0.4	**	**	**	***	*	**	ns	ns					
16:0	21.2 ± 0.7	21.1 ± 0.4	25.2 ± 1.5	23.4 ± 0.9	21.7 ± 0.21	18.9 ± 1.6	20.9 ± 1.7	23.2 ± 1.0	20.0 ± 1.1	19.8 ± 0.4	23.4 ± 1.5	23.3 ± 0.5	ns	ns	**	ns	ns	ns	ns	ns					
18:4ω3	1.4 ± 0.3	1.8 ± 0.4	1.0 ± 0.4	1.4 ± 0.2	1.6 ± 0.4	1.2 ± 0.6	1.3 ± 0.5	0.9 ± 0.4	0.6 ± 0.1	1.9 ± 0.3	1.4 ± 0.6	1.1 ± 0.2	ns	ns	ns	ns	ns	ns	ns	ns					
18:2ω6	2.7 ± 0.0	2.2 ± 0.4	1.4 ± 0.5	3.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.4	1.6 ± 0.1	3.0 ± 0.0	2.3 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.9 ± 0.1	ns	ns	ns	ns	ns	ns	ns	ns					
18:3ω3	0.8 ± 0.0	0.5 ± 0.2	0.6 ± 0.1	1.4 ± 0.0	0.7 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	1.0 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	1.1 ± 0.0	ns	ns	ns	ns	ns	ns	ns	ns					
18:1ω9c	15.0 ± 0.3	21.4 ± 0.1	22.0 ± 0.5	11.6 ± 0.8	13.8 ± 0.6	17.3 ± 2.0	16.6 ± 2.0	8.0 ± 0.4	12.8 ± 1.0	19.2 ± 0.3	17.7 ± 0.9	10.0 ± 1.3	ns	ns	ns	ns	ns	*	ns	*					
18:1ω7c	8.6 ± 0.4	10.5 ± 1.5	12.8 ± 1.2	5.7 ± 0.2	8.2 ± 0.7	10.3 ± 2.0	11.2 ± 1.5	6.7 ± 0.9	10.3 ± 0.9	10.8 ± 0.7	10.2 ± 1.1	6.1 ± 1.0	ns	ns	ns	ns	ns	ns	ns	ns					
18:0	1.4 ± 0.1	2.1 ± 0.3	2.5 ± 0.4	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.4	2.1 ± 0.4	1.4 ± 0.4	1.2 ± 0.3	1.5 ± 0.1	3.6 ± 1.5	1.9 ± 0.9	ns	ns	ns	ns	ns	ns	ns	ns					
20:1	-	1.0 ± 0.5	0.2 ± 0.2	0.5 ± 0.1	-	0.8 ± 0.3	0.5 ± 0.3	0.7 ± 0.1	-	0.7 ± 0.3	1.5 ± 1.0	1.0 ± 0.4	ns	ns	ns	ns	ns	ns	**	ns					
20:5ω3	16.8 ± 0.8	3.3 ± 0.3	3.7 ± 2.0	27.5 ± 0.9	17.3 ± 0.8	4.2 ± 0.3	5.8 ± 0.3	35.5 ± 2.0	19.4 ± 1.1	4.6 ± 1.1	6.0 ± 1.7	30.7 ± 2.8	ns	ns	ns	ns	ns	*	*	**					
22:6ω3	11.2 ± 1.1	2.2 ± 0.6	0.8 ± 0.8	16.1 ± 1.18	7.2 ± 0.3	1.3 ± 0.4	1.2 ± 0.7	10.7 ± 1.2	9.6 ± 2.3	2.4 ± 0.8	1.0 ± 0.6	13.6 ± 0.4	**	ns	ns	ns	ns	ns	ns	ns					
other	3.1	3.3	0.4	2.1	3.0	4.2	1.0	2.1	3.0	4.0	1.1	2.4													
16:1ω7c/16:0	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	1.0 ± 0.4	0.8 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	**	*	ns	ns	ns	*	ns	**					
18:1ω7c/18:1ω9c	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.3	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	ns	ns	ns	ns	ns	ns	ns	ns					
total ω7c	15.9	23.1	25.6	9.0	19.1	30.1	29.7	11.4	21.7	25.6	24.9	9.9	ns	***	ns	ns	ns	ns	ns	***					
total ω3	30.2	7.8	6.0	46.4	26.6	7.0	8.6	48.1	30.3	9.6	8.9	46.6	ns	ns	ns	ns	ns	*	*	ns					

"Other" includes categories less than 0.5% fatty acid in all three diets.

Most reports of the lack of ability to elongate and desaturate long chain ω 3 PUFAs are results from experiments with penaeid shrimps whose omnivorous diet is rich in long chain PUFAs. Morris and Sargent (1973) however, using labelled palmitic acid concluded that some oceanic crustaceans were capable of biosynthesis of higher polyunsaturated fatty acids. *Euphausia superba* may be capable of the elongation and desaturation of 18:3 ω 3 to 20:5 ω 3 and possibly to 22:6 ω 3.

The fatty acid profiles of the experimental animals in this study were compared with both the total lipid and the triacylglycerol fatty acid profiles from field and other laboratory samples (Fricke et al. 1984, Virtue and Green, unpublished data). The dietary influence would more likely be seen initially in the triacylglycerol profiles whereas the phospholipid profiles are usually conserved by their incorporation into cellular membrane. Fricke et al. (1984), found an increase in triacylglycerol PUFA levels of *Euphausia superba* from 1% 20:5 ω 3 and 0% 22:6 ω 3 in December to 3.3% 20:5 ω 3 and 0.7% 22:6 ω 3 in March in krill samples collected in the austral spring and summer. These percentages were marginally lower than the PUFA in the triacylglycerol fraction of all animals analysed in this study which averaged between 4.0% 20:5 ω 3 and 2.0% 22:6 ω 3. Experimental animals have maintained their total PUFA levels despite low levels available in the *Phaeocystis* diet. This may be evidence of the organisms ability to elongate and desaturate C₁₈ PUFA, however a mass balance of PUFA input and output together with labelling studies are needed to further elucidate fatty acid biosynthesis in *Euphausia superba*, and to determine specific biosynthetic mechanisms.

The monounsaturated fatty acid 18:1 ω 9c was higher in all lipid classes of krill fed exclusively on *Phaeocystis*. Significant differences were detected in the lipid classes between the prymnesiophyte and the diatom diets. This is a reflection of the high levels of 18:1 ω 9c found in *Phaeocystis* compared with the very low levels of this acid occurring in diatoms (Table 4.2 and 4.3) (Nichols et al. 1991). Significant differences were also detected for the monounsaturated fatty acid 16:1 ω 7c between the *Phaeocystis* and *Phaeodactylum* diets (Table 4.3). The interaction of these fatty acids between diets within lipid classes can be seen in Figure 4.2.

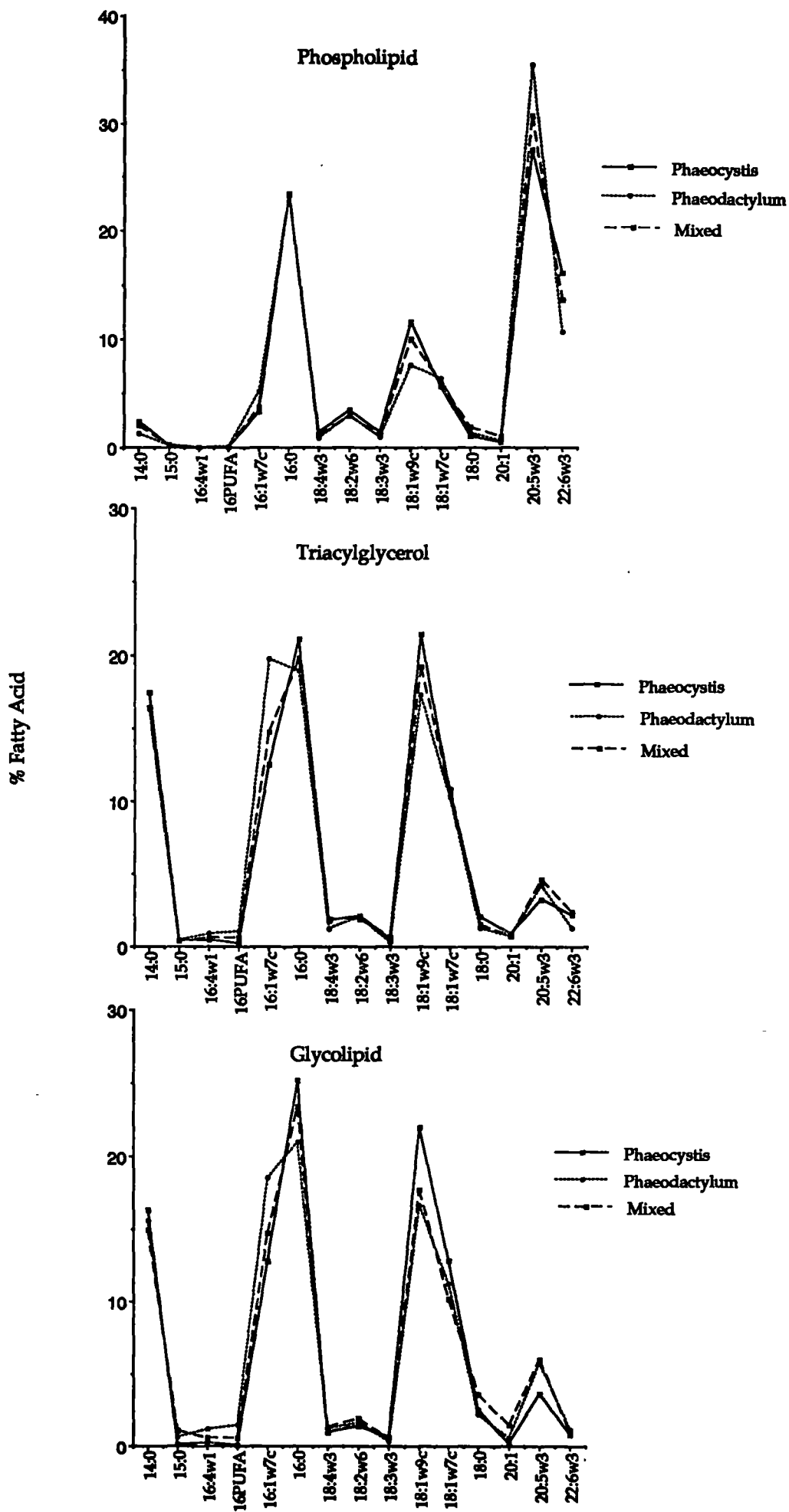


Figure 4.2 The interaction between % fatty acids in lipid fractions of *Euphausia superba* fed on *Phaeocystis*, *Phaeodactylum* and a mixed diet of these two phytoplankton.

The ratio of 16:1 ω 7c/16:0, which is usually greater than 1 in diatoms, was consistently highest in the lipid classes of the diatom only diet, lowest in the *Phaeocystis* diet and an average of the two in the mixed diet (Figure 4.3). These trends were found to be statistically significant (Table 4.3).

The isomer ratio 18:1 ω 7c/18:1 ω 9c of the total fatty acids ranged from 0.6 in the *Phaeocystis* fed krill to 0.8 in krill fed a mixed diet (Table 4.3), however there was no significant difference between diets or lipid classes. All phytoplankton analysed in this investigation had a ratio of less than 0.3. Dall et al. (1992) suggest that this ratio is a reflection of that found in the diet where as Clarke and Wickins (1980) indicate that very low or very high 18:1 ω 7c/18:1 ω 9c ratios may be dietary stress related.

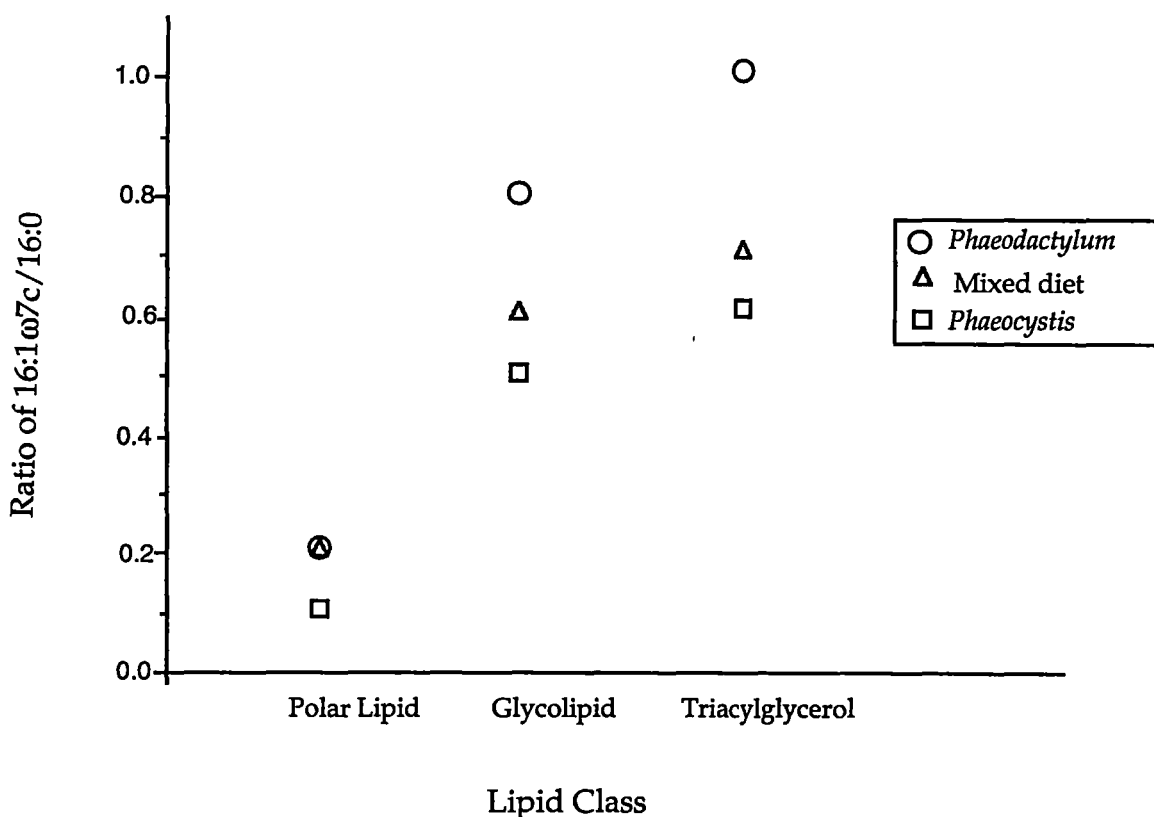


Figure 4.3 The ratio of 16:1 ω 7c/16:0 in lipid classes of krill fed diets of *Phaeocystis*, *Phaeodactylum* and a mixed diet.

Clarke (1980) found this ratio in the triacylglycerol fraction of ovary and male samples of *Euphausia superba* to be less than 1 and that in the total phospholipid fraction was usually greater than 1. He suggested the difference was due to the balance between exogenous supply and *de novo* synthesis of these fatty acids.

Knowledge of essential fatty acids and the ability and rate of PUFA biosynthesis of a particular species needs to be considered when exploring the use of biochemical signatures. No lipid compound is metabolically inert (Sargent and Whittle 1981) and the degree of catabolism of a potential marker may be highly variable between species. In this investigation the differences in fatty acids and the ratios of 16:1 ω 7c/16:0 and 18:1 ω 7c/18:1 ω 9c between *Euphausia superba* fed on different algal diets have implications in terms of lipid signatures. This biochemical information may provide reference to krill diet and feeding grounds.

4.4.4 Sterol composition of krill lipid

Total sterols ranged from 4.2 - 5.5 mg/g dry weight with cholesterol predominating (2.5 - 3.8 mg/g dry weight or approximately 4% of the total lipid). No significant differences were found in the krill sterols between diets. Although the relative level of cholesterol in krill (90-92%) did not differ significantly among the diets, significant differences were found in the neutral lipid fraction between the *Phaeocystis* and the *Phaeodactylum* diets (Table 4.4). These results differed to those of Casalta et al. (1984) who found lower levels of cholesterol (75-80%) in field *Euphausia superba* accompanied by a higher desmosterol (cholesta-5-24-dien-3 β -ol) level (15-17%). Brassicasterol (24-Methylcholesta-5,22E-dien-3 β -ol) the major sterol found in both *Phaeocystis* and *Phaeodactylum* (Volkman 1986, Nichols et al. 1991 and Virtue, unpublished data) was found to be a minor component (0.4 - 1.0 %) in all krill analysed. Cholesta-5,22E-dien-3 β -ol, although varying between diets, was detected in equally minor amounts. Desmosterol increased from 6.9% in the *Phaeocystis* fed krill to 8.2% in krill fed a *Phaeodactylum* diet (Table 4.4).

Table 4.4 Percentage sterol composition of *Euphausia superba* fed on *Phaeocystis*, *Phaeodactylum* and a mixed diet of these two phytoplankton: all data mean of triplicate analyses \pm SE. TL= Total lipid, TG= Triacylglycerol. Pc*Pd=*Phaeocystis* diet verses *Phaeodactylum* diet, Pc*Mx=*Phaeocystis* diet verses Mixed diet, Pd*Mx=*Phaeodactylum* diet verses Mixed diet. *P<0.05; **P<0.01; ***P<0.001

Sterol	ANOVA											
	<i>Phaeocystis</i> Diet		<i>Phaeodactylum</i> Diet		Mixed diet		Total Lipid			Triacylglycerol		
	TL	TG	TL	TG	TL	TG	Pc*Pd	Pc*Mx	Pd*Mx	Pc*Pd	Pc*Mx	Pd*Mx
Cholest-5-en-3 β -ol	90.4 \pm 3.0	91.2 \pm 1.3	90.5 \pm 0.7	83.9 \pm 3.4	92.0 \pm 0.5	86.5 \pm 0.7	ns	ns	ns	***	*	ns
Cholesta-5,24-dien-3 β -ol	6.9 \pm 2.8	6.5 \pm 0.4	8.2 \pm 0.3	13.4 \pm 2.7	7.5 \pm 0.4	12.1 \pm 0.6	ns	ns	ns	**	**	ns
Cholesta-5,22E-dien-3 β -ol	1.3 \pm 0.2	1.6 \pm 1.0	0.4 \pm 0.1	1.6 \pm 1.1	0.2 \pm 0.3	0.5 \pm 0.3	ns	ns	ns	ns	ns	ns
24-Methylcholesta-5,22E-dien-3 β -ol	1.4 \pm 0.4	0.7 \pm 0.1	0.9 \pm 0.3	1.1 \pm 0.3	0.4 \pm 0.3	0.9 \pm 0.1	ns	ns	ns	ns	ns	ns

Sterols are considered essential for growth and survival because crustaceans lack the ability to synthesise them *de novo* (Teshima and Kanazawa 1971, D'Abramo et al. 1985). Cholesterol is the starting product from which steroid hormones and the moulting hormones, ecdysone and ecdysterone are synthesised (Ponath and Adelung 1983).

Generally quite low levels of cholesterol are found in algae (Brown et al. 1989, Nichols et al. 1991). Although they cannot biosynthesise sterols, crustaceans have been reported to convert dietary phytosterols, via a desmosterol intermediate, to cholesterol (Teshima 1982). As biomarkers for krill feeding grounds the use of sterols or modifications of phytosterols, due to their exogenous origin, may have substantial potential. Further investigation however is required to characterise the biochemical capability of *Euphausia superba* with respect to sterol biosynthesis.

In summary a diet deficient in PUFAs did not induce a deficiency of these fatty acids in *Euphausia superba* under laboratory conditions. However this does not impute *Phaeocystis pouchetii* as a quality substrate but rather indicates *Euphausia superba*'s ability to bioconvert dietary fatty acids.

Euphausia superba stores lipid reserves as triacylglycerols rather than wax esters (Clarke 1984). Due to the absence of any substantial overwintering lipid storage they must therefore have the biochemical capabilities to exploit a myriad of food sources to sustain periods of reduced primary productivity. *Phaeocystis pouchetii*, in addition to being one of the first species to bloom in the austral spring and frequently dominating the sea-ice microalgal community (Garrison et al. 1987, Garrison and Buck 1989, Davidson and Marchant 1992b), has been reported as a major carbon source in open water during the austral winter (Ashworth et al. 1990). It is highly likely that, due to their apparent biochemical ability to adequately utilise the fatty acids of *Phaeocystis pouchetii* under laboratory conditions as demonstrated here, *Euphausia superba* may exploit this food source in the field.

Potential biomarkers for the use in comparative studies of *Euphausia superba* may include differences in the relative levels of fatty acids between diets and within specific lipid fractions, and the ratios 16:1 ω 7c/16:0 and 18:1 ω 7c/18:1 ω 9c highlighted in this investigation. To further clarify the viability of biomarkers in studies of *Euphausia superba*, a comparative analysis of field samples with their *in situ* diet should be undertaken in conjunction with further investigations of the biochemical abilities of *Euphausia superba*.

Addendum

The research in this paper was carried out by myself. Co-authors Peter Nichols, Stephen Nicol, Andrew McMinn and Liz Sikes played a supervisory role. All co-authors on this paper were my PhD supervisors.

Chapter 5. Reproductive trade-off in male Antarctic krill

Published in : Marine Biology, 1995 (in press).

Co-authors: P. D. Nichols, S. Nicol, and G. Hosie.

5.1 Abstract

Euphausia superba caught from a single swarm in Prydz Bay were kept alive on board the research vessel and subsequent natural mortality was monitored. Mortality was significantly higher for reproductive males (100% n= 68) than for females (3% n=186) within the first 3 days of capture. Total lipid and lipid class composition in male, female and juvenile *Euphausia superba* were analysed and compared. All reproductive male krill analysed from this swarm had low lipid levels (1-3% dry weight) with negligible triacylglycerol stores (0-2% of total lipid). Somatic lipid stores in female and juvenile krill ranged from 8-30% of which up to 40% was triacylglycerol. The fatty acid profile of the total lipids of reproductive male krill resembled that of a typical phospholipid fraction of krill. The algal sterols in the digestive gland of both male, female and juvenile indicate that all krill recently had been feeding. A decrease in reproductive male krill numbers in relation to body size was evident using sex ratios of krill swarms sampled over seven summers in Prydz Bay. There was a sharp decline in numbers of male krill once they attained a length of 51-55 mm. Low lipid levels accompanied by high mortality in reproductive male krill was attributed to reproductive costs.

5.2 Introduction

The cost of reproduction in most animals is attributed primarily to oocyte production by the female (Trivers 1972), although cases of male reproductive investment exceeding that of females are reported occasionally (Partridge and Farquhar 1981, Van Voorhies 1992). Biochemical and energetic costs of reproductive investment in males are not generally measured other than in terms of lifespan and survival.

A major component of the energy budget of the Antarctic krill population is the cost of reproduction, which to date is attributed solely to oocyte production (Quetin et al. 1994). During the short summer female krill utilise the increased phytoplankton production to produce up to ten of batches of lipid rich eggs (Ross and Quetin 1983, Ross and Quetin 1986). Although fecundity estimates vary, number of eggs per spawning episode has been reported to range from 100-14400 (reviewed by Miller and Hampton 1989). Females are capable of reproducing for up to five seasons (Ettershank 1983) and the energy cost of egg production has been estimated to be 6.39 kJ over the summer (190 days) (Clarke and Morris 1983). The total energy intake for female krill during the summer period was estimated to be 22.723 kJ compared to 15.228 kJ for male krill (Clarke and Morris 1983). The cost of sperm production and transfer is not accounted for in this budget.

Sperm are packaged into spermatophores that are transferred to females and fertilisation takes place when eggs are spawned. The energetic expense and behavioural sequence necessary for spermatophore transfer has not been investigated, primarily because krill have yet to be successfully bred in the laboratory. Information on male reproductive lifespan is unknown and it has been generally assumed that post spawning reproductive costs and energy losses incurred by sperm production in krill are insignificant (Clarke and Morris 1983, Miller and Hampton 1989).

The ultimate cost of reproduction to an organism is death. Many organisms do not survive after reproduction and classic examples include the Pacific salmon and the '17 year locust' (*Magicalicada septendecim*). Survival of the female only, after reproduction, although uncommon, has been reported in

various invertebrates. This reproductive strategy is rare in vertebrates with the only mammalian example being the Australian marsupial mice of the genus *Antechinus* (Braithwaite and Lee 1977).

Mortality after breeding in zooplankton has been inferred by skewed sex ratios of catch data. Post-reproductive changes in sex ratios favouring females have been reported in zooplankton species from polar regions (Mauchline and Fisher 1969, Wiborg 1971). Siegel (1987) reported that the male *Euphausia crystallorophias* has a shorter life span than the female. Latogurskij (1973) reported that male *Euphausia superba* suffered from higher mortality than females after spawning. Extensive observations of *Euphausia superba* were made by Marr (1962) during the 'Discovery era' in Antarctica who wrote:

"The swarms in general are composed of males and females in approximately equal numbers. In adult swarms however,subsequent to pairing, may in fact consist predominantly, and sometimes exclusively of females, all either gravid or spent, suggesting that when the males have transferred their spermatophores they begin to die off earlier than the females, the latter persisting for some time as the dominant, and eventually perhaps as the exclusive component of the swarm."

This hypothesis that male *Euphausia superba* suffer from higher mortality than females after reproduction is not generally accepted because of the extreme variability in sexual composition of swarms (Quetin and Ross 1984, Watkins 1986, Morris and Watkins 1986).

Lipids play a major reproductive role in krill. Lipid levels and lipid composition can be markedly influenced by sex and maturity stage. Reproductive female krill can have up to twice as much lipid as males (Clarke 1980, Seather et al. 1985). In this study the role of lipids in reproduction of male *Euphausia superba* was assessed. Mortality of reproductive krill was monitored under experimental conditions. Sex ratios of krill sampled in Prydz Bay from seven austral summers were analysed to investigate mortality associated with spawning in this area.

5.3 Materials and Methods

The majority of krill used in the reproductive study were caught on the January 27th, 1993 in the Prydz Bay region of Antarctica 66°30.83' S and 67°30.32' E (Figure 2.1) (as per Chapter 2 in the long term starvation study). Adult reproductive male krill from this single swarm were kept live and maintained individually in 2L containers on board ship. Live reproductive male krill were fed natural phytoplankton collected both using a plankton net tow or filtered from the on line sea water tap on board. All maintenance conditions and measurement procedures are described in section 2.3.1. Reproductive male krill caught during the 1991/92 and 1992/93 austral summers in the Prydz Bay region were also analysed for comparative purposes in this study.

Data used for the sex ratio figure (Figure 6.6) were collected by Graham Hosie and other members of the Australian Antarctic Division research program during the austral summers of 1981, 1984, 1985, 1987, 1991, 1992, 1993. Samples were collected in the Prydz Bay region (Figure 2.1) by both routine plankton hauls and aimed trawls at aggregations which were detected hydroacoustically (target trawls). Krill length was measured using Standard measurement 1 (Kirkwood 1982). Procedures for lipid extraction and analysis were the same as those described in Chapter 2.

5.4 Results and Discussion

Krill in this study were collected from a single swarm in which the majority of the krill in the swarm were either gravid or spawning females with attached spermatophore (approximately 80%). All reproductive male krill analysed from this swarm had low lipid levels (1-3% dry weight) with negligible triacylglycerol stores (0-2% of total lipid) (Figure 5.1).

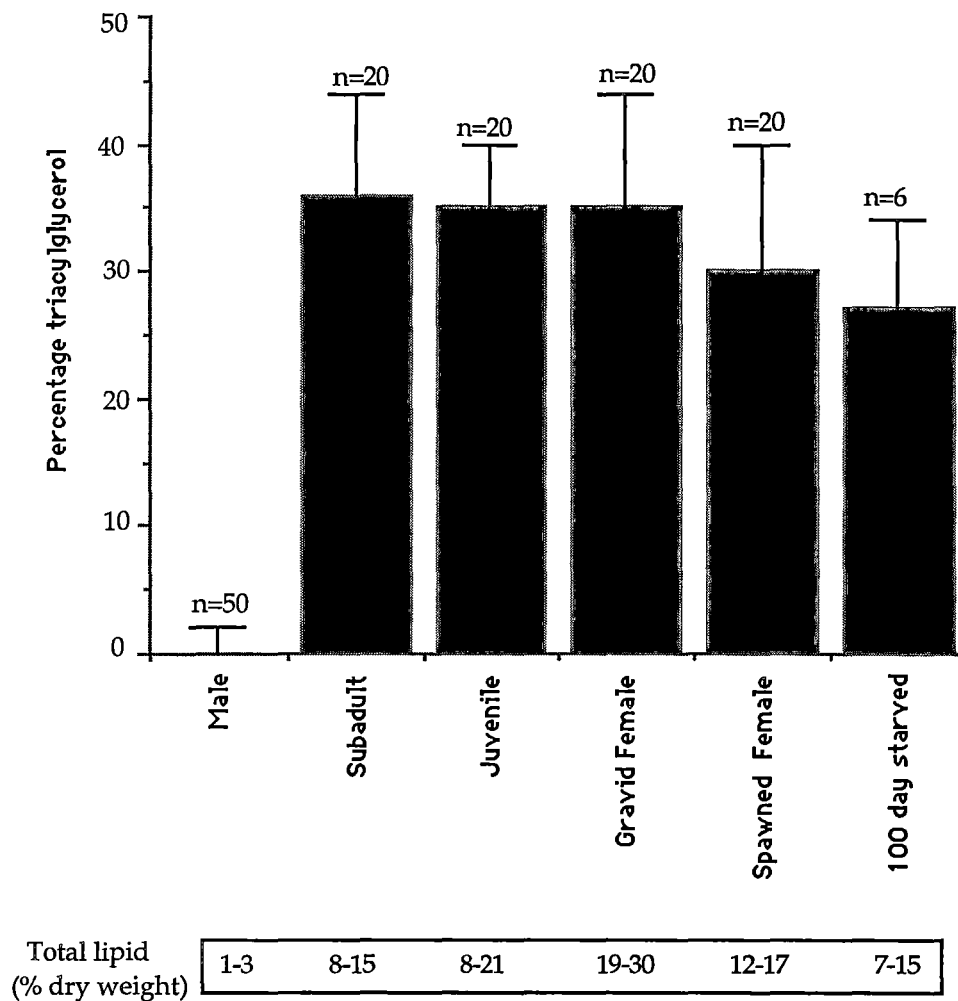


Figure 5.1 Comparison between male triacylglycerol (% of the total lipid dry weight) and total lipid levels (% dry weight of krill) with those found in female, juvenile and starved krill (see Chapter 2 for details for starved krill). Error bars = SD.

Lipid stores in female and juvenile krill ranged from 8-30% of which up to 40% was triacylglycerol (Figure 5.1). Lipid levels in female and juvenile krill were similar to data previously reported, however, male lipid levels in the present study were much lower (Pierce et al. 1969, Raymont et al. 1971, Ferguson and Raymont 1974, Clarke 1980, Clarke 1984, Saether et al. 1985, Hagen 1988).

For comparative purposes, male krill sampled from throughout the stations in Prydz Bay (Figure 2.1) over three austral summers (1991/92, 1992/93, 1993/94) were also examined. Lipids in male krill varied considerably with total lipid and triacylglycerol levels ranging from 1-25% and 0-40% respectively. There was a strong correlation between total lipid and triacylglycerol levels in male krill ($r^2=0.91$, $n=40$) (Figure 5.2). Males that had low levels of lipid (1-3%) accompanied by a depleted triacylglycerol store (0-2%) had presumably transferred spermatophores. Male krill that had high total lipid and triacylglycerol levels (up to 25% and 40% respectively) had obviously undergone spermatogenesis as indicated by their fully developed secondary sexual characteristics. However, these krill were probably only in the early stages of their reproductive life.

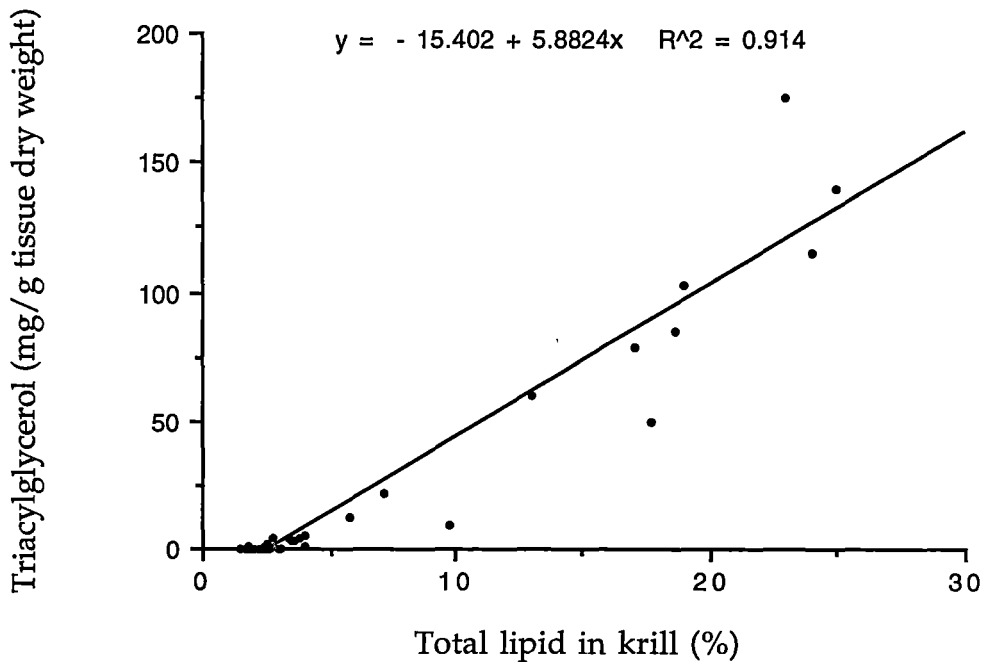


Figure 5.2 Correlation between triacylglycerol levels and total lipid in male krill caught over three summers (1991/92, 1992/93 and 1993/94) in the Prydz Bay region.

A depleted triacylglycerol store in most animals indicates starvation (Giese 1966). Triacylglycerol is an important energy reserve in crustaceans which accumulates during feeding and is depleted during starvation (Heath and Barnes 1970, New 1976). The inconsistency for Antarctic krill examined here lies with the fact that male krill, sampled during a period of phytoplankton abundance, had no triacylglycerol storage and low polar lipid levels (30 ± 4 mg/g dry wt). It could be inferred that these adult males were not eating for a substantial period prior to capture. However, male krill showed evidence of recent feeding. Males with depleted triacylglycerol stores had diatom fragments present in the gut (Figure 5.3) and sterols of algal origin in the digestive gland (Figure 5.4).

Sterols of algal origin in the digestive gland of krill indicate recent dietary composition and provide reliable indices of their nutritional condition (Chapter 4, Virtue et al. 1993b). Male krill with depleted triacylglycerol had similar levels of algal sterols in the digestive gland to those reported in krill with substantial triacylglycerol stores (Virtue et al. 1993b, Chapter 4, Figure 4.3) (Figure 5.4).

The fatty acid profile of male reproductive krill was typical of that reported in the phospholipid fraction of *Euphausia superba* (Virtue et al. 1993a, Chapter 4, Figure 4.3). Significant differences were found between male and other krill analysed in the present study in relative levels of both the long-chained polyunsaturated fatty acids (PUFA); eicosapentaenoic (EPA) and docosahexaenoic (DHA) (Table 5.1). These two PUFA comprised 55% of the total fatty acids found in male krill which was approximately double that found in all other animals. The percentage of total $\omega 7$ fatty acids in male krill was approximately half that found in other animals. Significant differences were found in the monounsaturated fatty acids; 16:1 $\omega 7c$ and 18:1 $\omega 9c$, the saturated fatty acid; 14:0, and the PUFA; 20:4 $\omega 6$ in male krill compared to all other krill analysed.

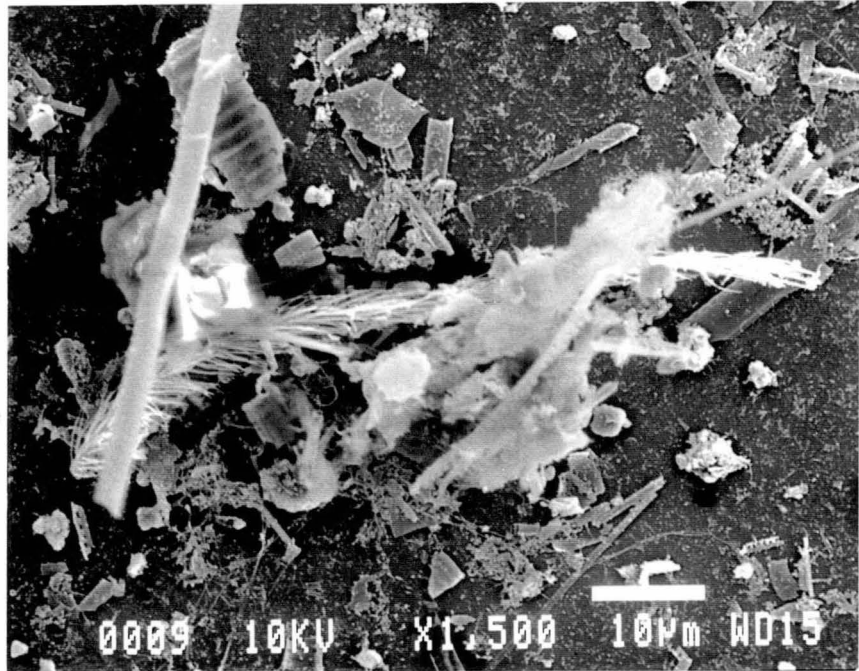
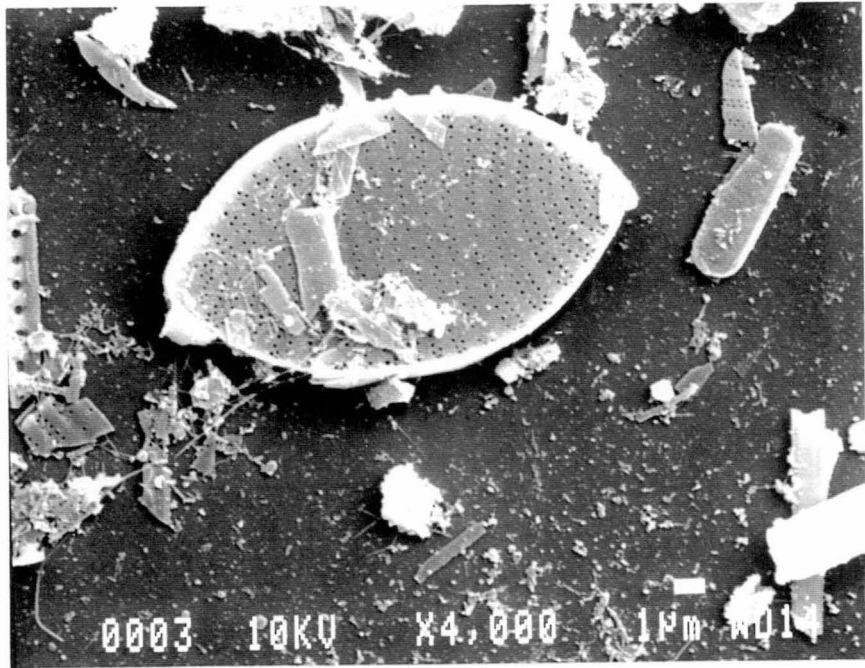


Figure 5.3 Electron micrographs showing diatom fragments in the stomach of freshly caught reproductive male krill with low total lipid and low triacylglycerol levels.

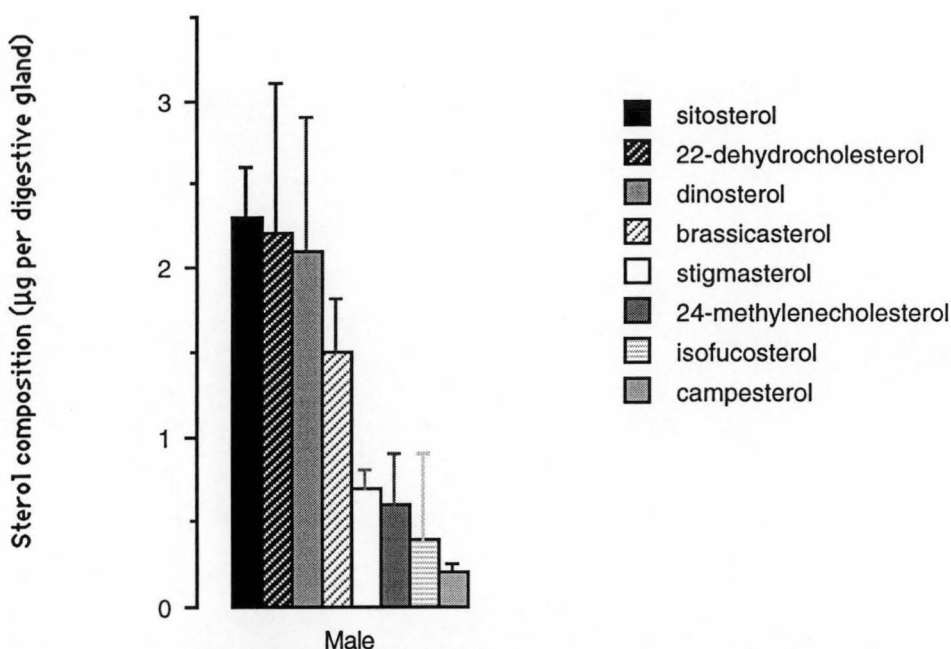


Figure 5.4 Algal sterols in the digestive glands of krill with low triacylglycerol stores (0-2%) immediately after capture. Data are means of eight analyses \pm SD.

Table 5.1 Percentage fatty acid of the total lipid of male, female (gravid and spent), and juvenile *Euphausia superba*. Data are the mean of three analyses \pm SD.

Percentage Composition				
Fatty Acid	Male	Gravid Female	Spent Female	Juvenile
14:0	1.3 \pm 0.3	10.4 \pm 1.1	9.5 \pm 1.1	10.3 \pm 2.2
16:1 ω 7c	1.3 \pm 0.1	9.5 \pm 0.2	9.9 \pm 1.6	7.0 \pm 2.3
16:0	17.2 \pm 0.9	20.7 \pm 0.9	20.8 \pm 0.8	21.7 \pm 0.3
18:4 ω 3	0.3 \pm 0.1	0.5 \pm 0.1	0.8 \pm 0.0	1.6 \pm 0.0
18:2 ω 6	2.7 \pm 0.2	2.7 \pm 0.6	2.3 \pm 0.4	3.2 \pm 0.6
18:3 ω 3	0.8 \pm 0.1	0.5 \pm 0.2	0.6 \pm 0.1	1.1 \pm 0.1
18:1 ω 9c	7.6 \pm 0.3	10.6 \pm 1.6	10.5 \pm 1.7	12.8 \pm 1.0
18:1 ω 7c	7.2 \pm 0.3	9.3 \pm 0.7	10.3 \pm 0.7	11.3 \pm 2.2
18:0	0.7 \pm 0.0	0.7 \pm 0.0	0.9 \pm 0.2	0.9 \pm 0.1
20:4 ω 6	1.5 \pm 0.1	0.0 \pm 0.2	0.5 \pm 0.2	0.7 \pm 0.1
20:5 ω 3	28.3 \pm 1.3	18.3 \pm 0.6	18.2 \pm 2.7	14.5 \pm 0.4
22:6 ω 3	26.3 \pm 0.4	8.8 \pm 0.5	9.5 \pm 1.3	9.0 \pm 0.2
Total ω 3	54.9	27.6	28.5	25.1
Total ω 7	8.5	18.8	20.2	18.3

The fatty acid profile of krill subjected to long term starvation has been reported for *Euphausia superba* (Virtue et al. 1995, Chapter 2, Table 2.2). The fatty acid profile of the male krill in the present study was significantly different from that found in starving krill (up to 115 days).

Relative levels of free fatty acids in the digestive gland of *Euphausia superba* increase during starvation (Virtue et al. 1993b, Chapter 3, Table 3.1). A rise in unesterified fatty acids during starvation is usually a result of the lipolysis of triacylglycerol which is a major energy yielding process (Stryer 1988). Free fatty acid levels in the digestive gland of male krill analysed in the present study were negligible.

The presence of algal sterols together with the fact that free fatty acids were not detected in the digestive gland indicate that the male krill were not starving. Depletion of triacylglycerol reserves in reproductive male krill therefore appears to be a reflection of the energetic cost of reproduction rather than a consequence of food limitation.

Mating involves substantial activity in male krill. Females sometimes carry several spermatophores which infers multiple pairing (Bargmann 1937). Most of the sperm mass may be lost during moulting (Harrington and Ikeda 1986). Remating may be required after moulting as moulting is continuous during the reproductive period (Nicol 1989). Although female krill lose substantial lipid reserves during spawning, the somatic lipid levels including triacylglycerol stores remained relatively constant throughout the reproductive period.

Mortality was dramatically higher in reproductive males (100%, n= 68) compared to that of females (3% n=186) in the swarm within the first 3 days of capture ($p < 0.001$). It is difficult to assess whether the stress of capture of male krill in an already poor physiological condition (depleted triacylglycerol store) was the cause of death or whether male krill died naturally. Recovery from a low lipid store may be unlikely in the field due to a lack of metabolic energy required to feed.

A decrease in reproductive male krill numbers in relation to body size was evident using sex ratio data on animals collected over seven austral summers in Prydz Bay (Figure 5.5). These samples were collected by both routine plankton hauls and aimed trawls at aggregations which were detected hydroacoustically (target trawls). There was a sharp decline in numbers of male krill once they attained a length of 51-55 mm. Males of this size are generally actively reproducing during the summer.

Changes in sex ratios within krill populations have been reported which suggest an association between mortality and breeding (Marr 1962, Mauchline and Fisher 1969, Mauchline 1980). Marr (1962) deduced, from the homogenous nature of post-spawning krill swarms which were devoid of males, that male *Euphausia superba* die immediately after spermatophore transfer. Both the mortality and biochemical data from the present study would affirm Marr's suggestion.

The cost of successful fertilisation places significant demands on the reserves of male krill. Spermatophore transfer appears to be responsible for the reduction in the lipid stores in male krill and this ultimately results in death. By channelling lipid reserves into reproduction in the short term, the ability to invest in future offspring would be reduced.

Death in both sexes after reproduction would be too costly for this species. It takes 2-3 years for krill to sexually mature (Hosie et al. 1988). Krill are subjected to a limited food supply for an extended period and hence growth is restricted to the short austral summer. An optimal reproductive strategy may be to limit the reproductive life of males only. An influx of new breeding males into the population every year increases the genetic diversity thereby enhancing the population's long term fitness. Reducing the overwintering population at a time when resources are limited, may also have a selective advantage in terms of an increased survival among juveniles and females. The preferential investment of resources in females at the expense of male krill may be a requisite trade-off to the success of *Euphausia superba* in the Southern Ocean ecosystem.

Addendum

The research in this paper was carried out by myself. Co-authors Peter Nichols and Stephen Nicol were my PhD supervisors. Graham Hosie contributed the data for Figure 5.5.

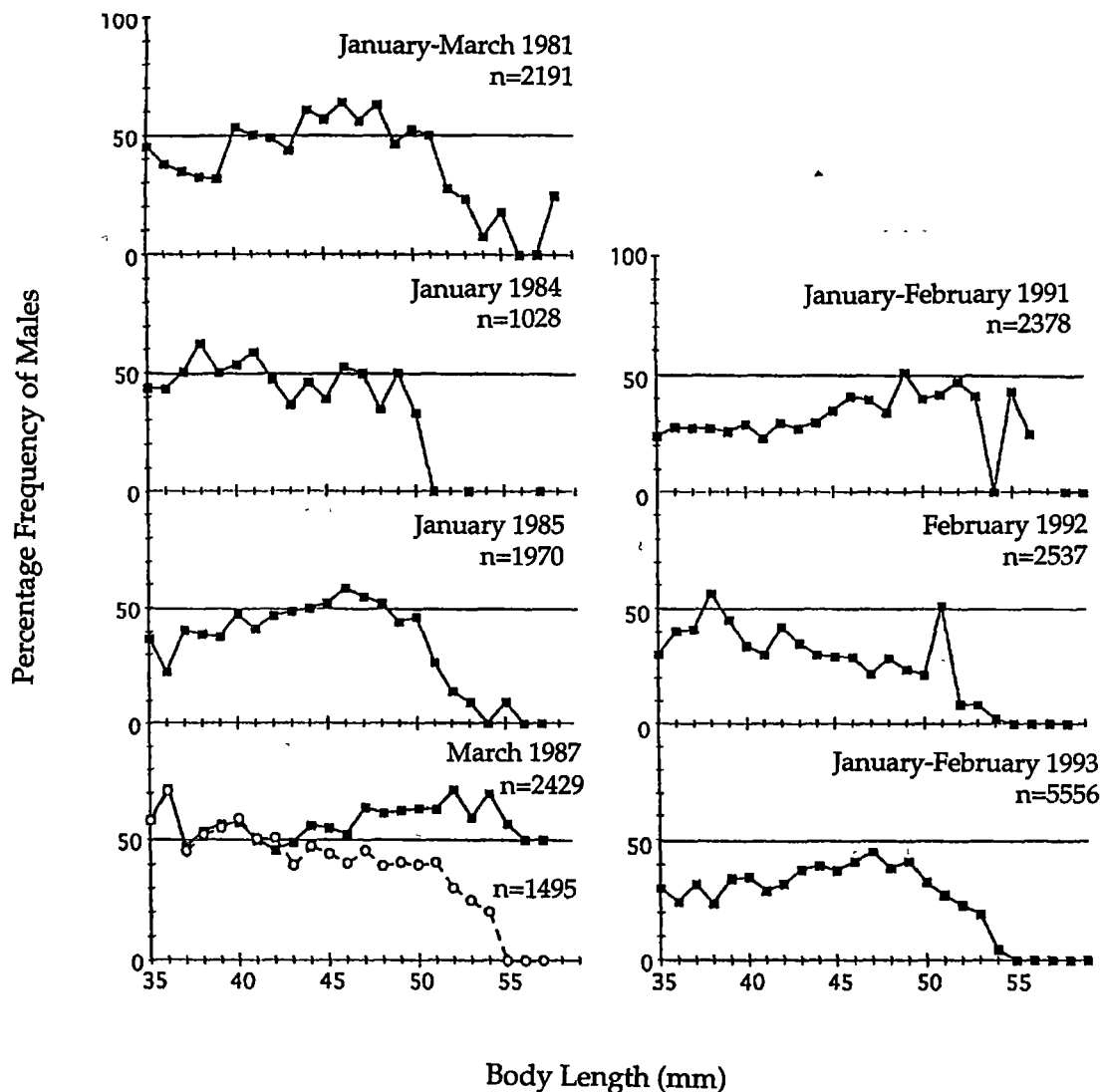


Figure 5.5 Percentage composition of males in the total sub-adult and adult population >35 mm. n= number of animals measured. 1:1 sex ratio represented by 50% line. The March 1987 sample comprises samples from all hauls (filled squares) and samples with target trawl removed (open circles). In this sample the target trawl comprised very large numbers of large (>46-48 mm) immature males collected from a superswarm (Hippinbottom and Hosie 1980)

Chapter 6. The biochemical composition of *Nyctiphanes australis* and its possible use as an aquaculture feed: lipids, pigments and fluoride content.

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Co-authors: R. E. Johannes, P. D. Nichols and J. Young.

6.1 Abstract

Nyctiphanes australis contained, on a dry weight basis, an average of 52% crude protein and 5.0 to 9.5% lipid. The fatty acid profile of *Nyctiphanes australis* was markedly unsaturated with a mean total $\omega 3$ fatty acid content of $48.6 \pm 2.4\%$ of total fatty acids. *Nyctiphanes australis* contained high levels of the essential long chain polyunsaturated fatty acids; eicosapentaenoic (EPA, 20:5 $\omega 3$) and docosahexaenoic (DHA, 22:6 $\omega 3$) ranging from 16.6 - 36.5% and 11.1 - 24.8% respectively. The concentration of total carotenoids ranged from 137 to 302 $\mu\text{g/g}$ dry weight, with no significant differences in concentration found with life stage or season. The carotenoids comprised 79.5% astaxanthin and 20.5% canthaxanthin. The lipid and pigment compositions of *Nyctiphanes australis* suggest the species could serve as a suitable feed for cultured salmonids. *Nyctiphanes australis*, like other euphausiids, was found to contain high levels of fluoride, with a seasonal range between 277 and 3507 $\mu\text{g/g}$ dry weight. High fluoride levels found in *Nyctiphanes australis* would not detract from its potential as a feed for salmonids because ingested fluoride is largely absorbed by the skeleton.

6.2 Introduction

Nyctiphanes australis is the principal euphausiid in continental shelf waters off south eastern Australia (Ritz and Hosie 1982). *Nyctiphanes australis* is the most important single food for a variety of abundant fishes in Tasmanian waters (O'Brien 1988). It was found, for example, to constitute 99.9% of the stomach contents of jack mackerel, *Trachurus declivis*, seined during a 19 month study (Webb 1976). These fish, in turn, support a commercial fishery of several tens of thousands of tonnes per year (Williams et al. 1987). Simple calculations suggest that this predator alone must therefore consume several tens of thousands of tonnes of krill per year.

Nyctiphanes australis is also the principal food of a number of seabird species, including the short-tailed shearwater, *Puffinus tenuirostris*, whose population numbers approximately nineteen million adults plus immature animals (Skira 1986). Taken together these observations suggest that the annual production of this euphausiid may exceed 100,000 tonnes in continental shelf waters off south eastern Australia.

Krill have been shown to be of high nutritional value when used as components of aquaculture feed (Storebakken 1988, Shimizu et al. 1990), and are used extensively in feed for farmed salmonids and other fishes in Japan (Odate 1979) and Canada (Sloan and Fulton 1987). An important biochemical characteristic in this connection is the high concentration of the polyunsaturated fatty acids (PUFA) in krill. PUFA are essential nutritional requirements of various commercially raised fish and shellfish, including salmonids (Lall 1991), however quantitative levels required by fish are not known. Fatty acid composition of fish is largely a reflection of that found in their diet (Cowey and Sargent 1972). High PUFA content in fish is commercially desirable as these fatty acids, in particular eicosapentaenoic acid (EPA, 20:5 ω 3) and docosahexaenoic acid (DHA, 22:6 ω 3), have been implicated in the prevention of human circulatory and other disorders (Bremner et al. 1989).

The carotenoid pigment, astaxanthin, is also typically present in high concentrations in krill (Funk and Hobson 1991). Astaxanthin and canthaxanthin are responsible for imparting the red colour to the flesh of salmonids and many other marine fish and shellfish species. Fish and other animals are unable to perform *de novo* synthesis of carotenoids, hence commercially farmed marine animals must obtain astaxanthin from their diets.

Tasmania supports a substantial salmonid farming industry which constitutes approximately 2% of the world production (A. Smithies, Salmonid Growers Association, personal communication). Due to the high local abundance of *Nyctiphanes australis*, the potential of this species as a constituent of fish feed in terms of lipid and astaxanthin content was investigated. Other comparable lipid studies of *Nyctiphanes australis* previously have been restricted to analysis of them as stomach contents of predator species (Cheah and Hansen 1970, Bishop et al. 1976).

Fluoride analysis of *Nyctiphanes australis* was also undertaken because other krill are known to have unusually high concentrations of fluoride in their exoskeletons (Nicol and Stolp 1989), which makes them unacceptable for direct human consumption. This has raised the issue of their appropriateness as food for animals raised for human consumption (Grave 1981).

6.3 Materials and Methods

Nyctiphanes australis were sampled monthly between November 1989 and January 1991 in Riedle Bay east of Maria Island off the Tasmanian east coast (Figure 6.1). Ring nets equivalent to one side of an Ocean Instruments 70 cm bongo net was used. Nets were cylindrical-conical with a 500 μm mesh aperture and an open area ratio of 5:1. Krill were sampled during the night and day at various locations along the 80 m depth contour. Additional sampling details are provided in Young et al. (1993).

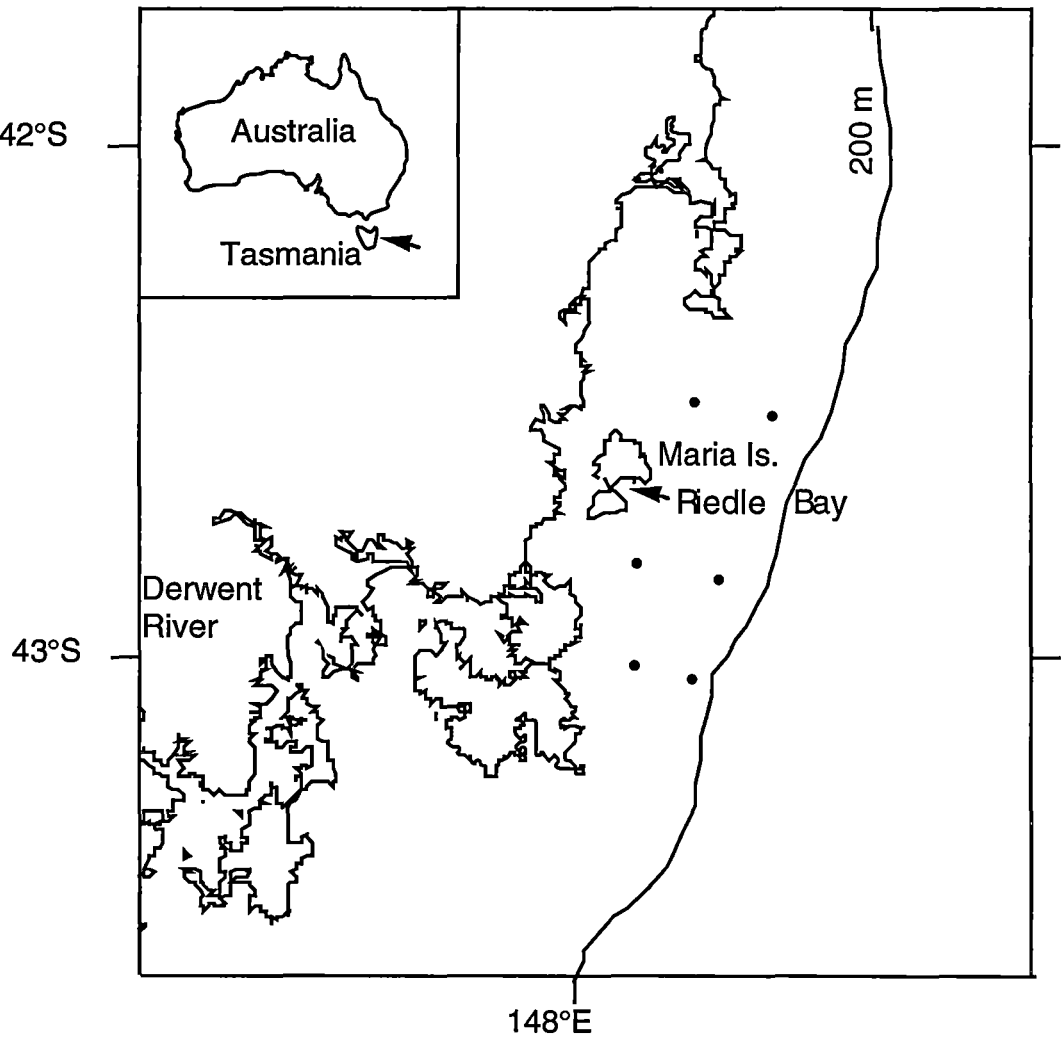


Figure 6.1 Location of sampling stations of *Nyctiphanes australis* (•) around eastern Tasmania.

Samples for chemical analyses were picked from the krill haul immediately after emptying the net. About 2 to 10 mg (dry weight) of krill were used for each analysis (water content of krill ranged between 75% and 83% (n=12) and a mean of 79.33% was used to convert wet weights to dry weights). Within minutes of capture, samples of krill were placed in vials and stored in liquid nitrogen. On return to the laboratory samples were transferred to a storage dewar containing liquid nitrogen and remained there until analysed. Individual samples were invariably dominated by krill within a narrow size range. Sizes varied considerably between samples however, and are reported as adults > 10 mm, subadults 5 -10 mm and juveniles < 5 mm.

6.3.1 Biochemical analysis

Procedures for lipid extraction and analysis are the same as those described in Chapter 2.

Protein analysis

A sample of mixed krill (*Nyctiphanes australis*) (0.61 g wet weight) using animals of different stages collected from various times of the year was analysed for protein. Total nitrogen was assayed by the Kjeldahl method (AOAC 1984). Wet weight was converted to dry weight using a mean water content of 79.33%.

Pigment analysis

Total carotenoid content was estimated spectrophotometrically; a portion of the total lipid extract in 90% acetone was analysed for pigment composition using a Shimadzu UV-240 spectrophotometer (300-700 nm).

A representative sample of mixed krill using animals of different stages collected from various times of the year was analysed using HPLC (Spectraphysics SP8800 ternary pump, SP8792 column heater, and Spherisorb ODS/2 column (25 cm X 4.6 mm I.D.). Samples were injected using a Gilson 231 autosampler which mixed 120 µl extract with 30 µl water

and injected 125 µl of the resultant mixture (Wright et al. 1991). The autosampler rack had been modified so samples were maintained at -20°C until ready for injection. The solvent system used was that of Wright et al. 1991:

A: Methanol: Ammonium acetate buffer (pH 7.1, 0.5M), 80:20 (v/v).

B: Acetonitrile : Water, 90:10 (v/v).

C: Ethyl Acetate.

All solvents were HPLC grade and filtered before use. Solvents were continually degassed by helium sparging.

Data was collected and integrated using Spectrafocus and Waters Maxima software. In the krill sample two peaks were resolved and identified by co-chromatography. One peak matched both the maximum wavelength (473 nm) and retention time (12 mins) of the astaxanthin standard. The other peak co-chromatographed with known canthaxanthin present in the blue green algae, *Anabaena flos-aquae* with a maximum wave length of 467 nm and a retention time of 8 mins.

High resolution MS data of the astaxanthin standard was acquired using a Kratos Concept ISQ with a probe inlet. Accurate mass data was acquired at 10,000 resolution at a scan speed of one second per decade using perfluorokerosene as an internal reference. The standard was determined to contain pure astaxanthin.

Fluoride analysis

Fluoride was liberated from freeze dried krill samples in a petri dish by acid digestion using perchloric acid saturated with AgSO₄. The fluoride from this digest diffused into the petri dish lid which was coated with ethanolic sodium hydroxide solution (4% wt/vol. NaOH in 90% EtOH) while being heated at 50°C for 16 hours. The alkaline mixture was dissolved in a 1:1 solution of water and TISAB II buffer consisting of NaCl and cyclohexylenedinitrilo tetraacetic acid in glacial acetic acid (CDTA), and water. The pH was adjusted to between 5.0 and 5.5 with 20% NaOH (Lewis et al. 1987). Fluoride concentration was measured with an Orion Model 96-09 combination fluoride electrode and a Radiometer Ion 85 pH/mV meter (resolution 0.1 mM).

6.4 Results

The average crude protein content of *Nyctiphanes australis* was 52% of total dry weight. The lipid content of *Nyctiphanes australis* ranged from 5.1% to 9.6% of total dry weight and no statistical difference was found between season or life stage. The lipid composition was dominated by polar lipids (including phospholipids and glycolipids) ranging from 68 to 86%. Triacylglycerols and sterols ranged between 5 to 21% and 5 to 8 % respectively (Table 6.1). No significant seasonal differences were detected in the lipid class composition of samples.

Cholesterol was the major sterol with an average concentration over life stage and season of 1.9 ± 1.0 mg/g dry weight. Only traces of desmosterol (cholesta-5, 24-dien-3 β -ol) and brassicasterol (24-methylcholesta-5,22E-dien-3 β -ol) were detected. Non-esterified (free) fatty acids were only minor components of the total lipid ($3.8 \pm 1.1\%$, Table 6.1). This relatively low percentage of free fatty acid indicates adequate storage of the samples prior to extraction (Saether et al. 1986).

The saturated fatty acid 16:0 and the long chain polyunsaturated fatty acids; eicosapentaenoic (EPA, 20:5 ω 3) and docosahexaenoic (DHA, 22:6 ω 3) dominated the fatty acid composition of *Nyctiphanes australis* (Table 6.2). The two major polyenoic acids, EPA and DHA, ranged from 16.6 to 36.5% and 11.1 to 24.8% of total fatty acids, respectively. The fatty acid profile was markedly unsaturated, with the mean total ω 3 fatty acids being $48.6 \pm 2.4\%$. Significant differences were found in the levels of EPA and DHA between life stages. Both adult and subadult life stages differed significantly from juvenile krill in these two PUFAs. Juveniles were found to have a higher mean percent EPA (23.6%) than the adult (17.7%) and sub adult (19.5%). Juveniles had lower mean percent DHA (18.1%) compared with adults (22.3%) and subadults (23.7%). These differences were probably due to the influence of one sample (Sept-90) which was significantly different from the total data set. No significant differences were found, however, in the total ω 3 fatty acids between life stages and season.

Table 6.1 Dry weight, lipid class, total lipid, lipid class ratios, cholesterol, carotenoid and fluoride levels of *Nyctiphanes australis* as a function of season and life stage. (A adult, SA subadult, J juvenile).

	1989				1990										Mean (SD)	
	Nov	Dec			Feb	Mar					Apr	May	Jun	Sep		Nov
	A	J	A	A	J	J	SA	A	A	A	SA	A	A	J		A
Krill sample (g dry wt)	0.05	0.11	0.02	0.02	0.05	0.02	0.03	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.04	0.04 (0.03)
Lipid class (mg g ⁻¹ dry wt)																
triacylglycerol	12.2	5.3	9.8	4.8	6.2	9.9	8.8	14.9	11.4	14.0	10.9	16.1	4.1	14.0	14.6	11.2 (4.1)
free fatty acid	0.0	2.2	2.7	2.4	4.3	2.8	2.6	3.7	2.1	0.0	2.7	4.0	3.4	2.3	3.1	2.8 (0.8)
polar lipid	39.5	39.5	66.2	58.1	52.6	52.1	48.4	69.9	58.0	70.1	55.8	61.1	60.8	63.2	48.0	64.1 (10.0)
sterol	4.5	4.0	4.3	3.9	5.0	4.7	4.4	6.1	5.2	5.8	5.4	5.6	4.1	5.9	4.7	5.5 (0.7)
Total lipid (mg g ⁻¹ dry wt)	56.2	50.9	83.0	69.3	68.1	69.6	64.1	94.6	76.7	89.9	74.8	86.8	82.4	85.4	70.4	84.7 (11.4)
Cholesterol (mg g ⁻¹ dry wt)	1.1	0.4	3.9	2.0	1.1	0.3	1.9	2.4	2.4	2.4	2.4	2.4	0.8	2.6	1.9	1.9 (1.0)
Percentage lipid composition																
triacylglycerol	15.7	10.3	11.8	7.0	9.1	14.3	13.7	15.8	14.9	15.6	14.5	18.5	5.0	16.4	20.7	14.7 (4.3)
free fatty acid	0.0	4.3	3.3	3.5	6.4	4.1	4.0	3.9	2.7	0.0	3.6	4.6	4.1	2.7	4.4	3.8 (1.1)
polar lipid	78.5	77.6	79.7	83.9	77.3	74.8	75.4	73.9	75.7	77.9	74.5	70.4	86.0	74.0	68.1	87.1 (4.5)
sterol	5.8	7.8	5.2	5.6	7.3	6.8	6.9	6.4	6.8	6.5	7.3	6.5	5.0	6.8	6.7	7.5 (0.8)
Total lipid (% dry wt)	7.8	5.1	8.3	6.9	6.8	7.0	6.4	9.5	7.7	9.0	7.5	8.7	8.2	8.5	7.0	8.5 (1.1)
Triacylglycerol:polar lipid	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.3	0.2 (0.1)
Free fatty acid:polar lipid	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.5 (0.1)
Total carotenoid ^a																
µg g ⁻¹ dry wt	161	151	196	169	178	174	137	303	186	140	263	252	206	200	146	215 (46)
µg g ⁻¹ lipid dry wt	2080	2959	2361	2432	2616	2500	2130	3202	2420	1555	3517	2904	2494	2342	2068	2895 (489)
Fluoride (µg g ⁻¹ dry wt)	531	548	1079	1084	684	3507	277	1198	665	2521	3212	1077	3500	1930	883	1556 (1129)

^a Carotenoid included both astaxanthin and canthaxanthin at ≈ 79.5 and 20.5%, respectively.

Table 6.2 Percentage fatty acid composition of *Nyctiphanes australis* as a function of season and life stage. (A adult, SA subadult, J juvenile, "other" includes all categories < 0.5%).

Fatty Acid	1989				1990										Mean(SD)	
	Nov	Dec			Feb	Mar					Apr	May	Jun	Sep		Nov
	A	J	A	A	J	J	SA	A	A	A	SA	A	A	J		A
14:0	5.5	4.9	5.3	4.2	4.4	6.1	5.6	5.9	5.4	5.8	5.8	6.2	3.5	3.5	6.0	5.2 (0.9)
15:1ω8	0.1	0.1	0.2	0.0	0.2	0.2	0.3	0.0	0.3	0.4	0.0	0.0	0.7	0.0	0.0	0.2 (0.2)
15:0	1.1	1.2	0.9	0.5	1.3	1.2	1.0	1.0	1.0	1.7	1.0	1.5	1.2	0.3	0.9	1.1 (0.3)
16:4ω1	0.6	0.4	0.6	0.5	0.4	0.5	0.3	0.4	0.3	0.5	0.5	0.8	0.5	1.3	0.4	0.5 (0.2)
16:3ω4	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.4	0.0	0.1	0.2	0.6	0.1	0.2 (0.1)
16:1ω7	2.6	4.3	2.7	2.6	2.6	2.9	1.6	1.7	1.7	2.3	2.2	2.2	2.1	7.0	2.5	2.7 (1.4)
16:1ω5	0.7	0.4	0.7	0.3	0.4	0.4	0.3	0.3	0.2	0.6	0.3	0.1	0.4	0.5	1.0	0.4 (0.2)
16:0	25.2	24.8	22.5	21.3	23.0	23.1	21.6	21.2	20.9	23.4	21.4	20.9	23.5	20.2	21.4	22.3 (1.5)
17:0	0.9	0.9	0.8	0.5	1.0	1.0	0.9	0.9	0.9	1.2	0.8	1.1	1.1	0.4	0.9	0.9 (0.2)
18:4ω3	5.1	2.7	5.5	3.1	2.9	4.3	2.9	4.7	3.5	2.6	4.2	5.3	4.0	1.8	7.3	4.0 (1.4)
18:2ω6	3.6	3.0	3.5	2.5	3.2	3.5	3.5	4.1	3.5	3.5	4.0	3.2	3.0	1.2	2.8	3.2 (0.7)
18:3ω3	3.8	2.3	3.2	2.0	3.4	3.1	2.5	3.7	2.6	3.2	3.5	3.4	3.2	0.5	3.0	2.9 (0.8)
18:1ω9	6.5	6.2	6.8	6.8	7.9	7.0	8.6	8.2	8.2	7.9	8.5	7.3	7.8	5.3	5.6	7.2 (1.0)
18:1ω7	3.2	3.9	3.3	3.8	3.2	3.3	2.6	2.8	2.7	3.8	3.1	3.6	3.3	5.8	2.9	3.4 (0.8)
18:0	2.3	2.3	2.2	1.6	2.9	2.7	2.2	2.2	2.3	2.3	2.1	2.1	2.7	2.2	1.6	2.2 (0.4)
20:4ω6	0.0	0.0	0.0	2.5	0.0	0.0	0.0	1.8	2.3	1.9	1.8	0.0	1.9	0.0	0.0	0.8 (0.3)
20:5ω3	17.1	20.9	17.7	21.3	19.3	18.8	20.6	16.6	18.4	16.6	17.1	17.5	16.7	36.5	17.9	19.5 (4.9)
20:4ω3	0.5	0.4	0.5	0.3	0.5	0.3	0.2	0.4	0.2	0.4	0.3	0.3	0.4	0.5	0.6	0.4 (0.1)
20:2ω6	0.3	0.2	0.2	0.1	0.3	0.2	0.2	0.6	0.1	0.3	0.0	0.2	0.0	0.0	0.2	0.2 (0.1)
20:1ω9	0.5	0.7	0.4	0.5	0.6	0.4	0.3	0.2	0.1	0.4	0.3	0.1	0.7	0.5	0.9	0.5 (0.2)
22:6ω3	18.9	19.2	21.8	24.8	21.3	20.6	24.4	22.4	24.4	20.5	22.9	23.0	22.0	11.1	22.6	21.3 (3.3)
22:5ω3	0.4	0.4	0.5	0.3	0.4	0.3	0.4	0.5	0.3	0.4	0.2	0.5	0.6	0.8	0.5	0.4 (0.1)
other	0.5	0.4	0.3	0.0	0.5	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.2	0.0	0.4	0.2 (0.1)
16:1ω7/16:0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1 (0.1)
18:1ω7/18:1ω9	0.5	0.6	0.5	0.6	0.4	0.5	0.3	0.3	0.3	0.5	0.4	0.5	0.4	1.1	0.5	0.5 (0.2)
total ω7	5.8	8.3	5.9	6.4	5.8	6.1	4.2	4.5	4.3	6.1	5.2	5.7	5.4	12.8	5.4	6.1 (2.1)
total ω3	45.8	45.8	49.3	51.8	47.9	47.4	51.0	48.2	49.5	43.7	48.2	50.0	46.9	51.2	52.0	48.6 (2.4)

Both astaxanthin and canthaxanthin were detected in *Nyctiphanes australis* with total carotenoid levels ranging from 137 to 303 µg/g dry weight. Levels of astaxanthin and canthaxanthin were approximately 79.5% and 20.5% respectively. Fluoride concentrations ranged between 277 to 3507 µg/g dry weight. No significant differences in either carotenoid or fluoride levels were evident between season or life stage (Table 6.1).

6.5 Discussion

Marine zooplankton tend to have lower lipid concentrations at lower latitudes (Sheard 1953), and *Nyctiphanes australis* is no exception. The total lipid in *Nyctiphanes australis* (approximately 8.5% dry weight) was less than values which characterise high latitude krill species. *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis* for example, all of which are found in northern polar regions, reached seasonal peak lipid contents of about 40, 40 and 50% dry weight respectively (Falk-Petersen et al. 1981); concentrations varied seasonally roughly threefold, fourfold and fourfold respectively (Saether et al. 1986). Lipid levels of the Antarctic euphausiid, *Euphausia crystallorophias*, ranged between 10% dry weight in November and 35% dry weight in May (Littlepage 1964). All these polar species depend on lipid stores to survive periods of low plankton production in the winter. Lipid levels of the Antarctic krill *Euphausia superba*, collected between spring and autumn are also highly variable and range from 3-36% dry weight (Clarke 1980, Ellingsen and Mohr 1981, Saether et al. 1986, Hagen 1988, Virtue et al. 1993a). It has been proposed that this species may not rely solely on lipid sources for overwintering (Ikeda and Dixon 1982). Unlike high latitude species, little seasonal variation in lipid content was detected in *Nyctiphanes australis* in this study.

All samples of *Nyctiphanes australis* analysed in this study had relatively low levels of the storage lipid, triacylglycerols (14.7%), compared to other euphausiids such as *Meganyctiphanes norvegica* (53%), *Thysanoessa raschii* (44%) and *Euphausia superba* (37%) (Sargent and Falk-Petersen 1981, Virtue et al. 1993a). The majority of lipid in *Nyctiphanes australis* was in the form of structural components. Phospholipids and cholesterol are structural

elements of the plasma membrane whereas storage lipid such as triacylglycerols and wax esters are used as energy stores (Lee et al. 1970, Sargent 1976).

The similarity in lipid content and composition of *Nyctiphanes* sampled throughout November 1989 to November 1990 would imply a relatively constant available food supply throughout our sampling period. Harris et al. (1987) however, reported seasonal fluctuations in phytoplankton in this region with blooms occurring during spring. *Nyctiphanes australis* is omnivorous (Mauchline 1980) and Ritz et al. (1990) concluded from a study on the stomach contents analysed quantitatively throughout the year, that *Nyctiphanes australis* was an opportunistic omnivore/detritivore. Young et al. (1993) reported no significant relationship between length and weight of *Nyctiphanes australis* with season using samples collected from the same stations as those of the present study. Due to an ability to exploit a variety of food sources *Nyctiphanes australis* is able to maintain physiological status in terms of lipid content and composition under reduced phytoplanktonic conditions.

Although physiological condition appears to be maintained in *Nyctiphanes australis* throughout the year, a response to environmental conditions may be evident in a more dynamic sense. Young et al. (1993) reported significant variation both seasonally and interannually in density, biomass and population structure of *Nyctiphanes australis*. This variation was attributed to regional oceanographic conditions driving phytoplankton production. Biomass estimates may also be influenced by both seasonal predation upon *Nyctiphanes australis* and the fact that this species may be living at depth out of plankton tow range during winter months (Young et al. 1993).

In terms of a mariculture feed, the total lipid level of *Nyctiphanes australis* is near the optimum range used for salmonids. Fish and crustaceans fed diets high in total lipid accumulated excess lipid which was deposited as visceral fat. This excess is discarded as waste during processing. The weight of the mesenteric adipose tissue in salmonid fish has been found to increase with increasing dietary lipid (Lin et al. 1977, Takeuchi et al. 1978).

A number of samples analysed which were not included in this data set contained wax esters ranging from 2 to 7% of their total lipid. All these samples consisted of very small individuals. Microscopic examination of these samples revealed that they also contained significant numbers of copepods, unlike samples consisting of large euphausiids, which could be readily sorted. Analyses for samples containing copepods are not presented here. These observations however, may explain previous conflicting reports concerning the presence or absence of wax esters in *Nyctiphanes australis*; Bishop et al. (1983) reported significant concentrations in this species, whereas Cheah and Hansen (1970) found none.

Total ω 3 fatty acids constituted almost half the total fatty acids found in *Nyctiphanes australis* with the two essential polyenoic acids, EPA and DHA dominating. Levels of these two PUFA compared well with those found in partially digested krill from stomachs of slender tuna (Bishop et al. 1976). Levels of both EPA and DHA were approximately 25-30% higher than those reported in polar species such as *Meganyctiphanes norvegica*, *Thysanoessa raschii*, *Thysanoessa inermis* (Sargent and Falk-Petersen 1981) and *Euphausia superba* (Virtue et al. 1993a). The food source of *Nyctiphanes australis* may have substantial quantities of both eicosapentaenoic and docosahexaenoic acids. Alternatively, or in addition, *Nyctiphanes australis* may possess some ability to elongate and desaturate dietary fatty acids.

The superiority of long chain over short chain C_{18} PUFA in maintaining growth has been reported in nutritional studies for marine fish (reviewed by Sargent and Whittle 1981). It has been suggested that carnivorous marine fish, which have natural diets high in DHA and EPA, may not have evolved or perhaps have lost the enzymatic pathways for the elongation and desaturation of shorter-chain PUFA to these longer ones (Cowey and Sargent 1977, Sargent and Whittle 1981).

Omega 3 fatty acids are essential for salmonid development (Ashton et al. 1993). Species such as rainbow trout (*Oncorhynchus mykiss*) require ω 3 rather than ω 6 fatty acids (Castell et al. 1972). Dietary deficiencies in ω 3 fatty acids in salmonid fish causes physiological dysfunction of developing fish and early embryonic mortality (Watanabe 1982, Leray et al. 1985). Because *Nyctiphanes australis* contains very high levels of long-chain omega 3 PUFA it would therefore be an attractive food source for mariculture species in this regard.

Crude protein content of *Nyctiphanes australis* (52%) was typical of that found in euphausiids (reviewed by Mauchline 1980). Crude protein is not a measure of metabolizable energy. Krill carapace which is a nitrogenous polysaccharide represents a portion of crude protein. Amino acid protein constitutes 78% of the crude protein in whole *Euphausia superba* (Pierce et al. 1969 and Siebert et al. 1980). Amino acid content was not analysed in *Nyctiphanes australis* in the present study, however assuming similar levels to that found in *Euphausia superba*, protein in terms of metabolizable energy would be about 40%. A survey of the quality of various fish meals available to the Tasmanian salmonid industry reported percentage protein in the range of 60 to 70% (Foster 1991). The protein content of *Nyctiphanes australis* would be considered adequate in terms of a mariculture feed although supplemental protein may be required.

Although the fluoride concentration in whole *Nyctiphanes australis*, like fluoride concentrations in other euphausiids, would be considered too high for both human consumption and stock feed, this species would be suitable for mariculture feed. The United States Food and Drug Administration (USFDA) allowance for human consumption is 100 µg/g of fluoride per day (Budzinski et al. 1985). In both vertebrates and invertebrates fluoride is accumulated in the skeletal structures. Grave (1981) reported that the elevated fluoride concentration in salmonids fed a pure krill diet was restricted largely to skeletal tissue. Fluoride content of muscle tissue increased slightly but did not exceed concentrations reported in various wild salmonid species and was less than 2% of the USFDA limit for human consumption. Oehlenschläger and Manthey (1982) investigated the fluoride content of Antarctic fish and no differences were found in fish feeding on krill and fish feeding predominantly on other fish. They found fish muscle to contain approximately 2 µg/g fluoride which is similar to fish in other waters, however the fluoride level found in bone tissue of these fish was in the order of 600-1200 µg/g. The flesh of mariculture species such as salmonid fed on a krill based diet would not therefore be expected to contain high levels of fluoride. Rather fluoride if accumulated, might be expected to be found in the skeletal material.

A large variation in fluoride levels in *Nyctiphanes australis* was found in this study. Adelung et al. (1987) reported over 99% of fluoride

concentrated in the cuticle of euphausiids (2600 µg/g dry weight in *Euphausia superba* and 3300 µg/g in *Meganyctiphanes norvegica*). As fluoride accumulates mainly in the chitinous exoskeleton of crustaceans and is found in very low concentrations in the muscle and soft tissue (50-100 µg/g) (Szewielow 1981), a substantial fluctuation within the moult cycle would be expected in *Nyctiphanes australis*.

Astaxanthin is the major carotenoid in many crustaceans and was found to be the dominant carotenoid in *Nyctiphanes australis* in this study.

Nyctiphanes australis is somewhat richer in this pigment than *Euphausia superba* which was found to have a mean astaxanthin content of approximately 94 µg/g dry weight (converted from wet weight using a 77% tissue water content for this species) (Clarke 1980). Carotenoids such as astaxanthin and canthaxanthin are used as pigmenting agents by the mariculture industry. Carotenoids cannot be synthesised *de novo* by salmonids and hence dietary supplements are required in net pen reared fish (Storebakken and No 1992).

Euphausia superba, which is rich in carotenoids and contains mainly (3R,3'R)-astaxanthin diester, was used successfully to enhance integument pigmentation of cultured yellow tail and sea bream (Fujita et al. 1983, Maoka et al. 1985, Miki et al. 1985). Shigeru et al. (1987) fed oil extracted from *Euphausia superba* to 180 g coho salmon (*Oncorhynchus kisutch*) for 8 weeks and reported marked flesh pigmentation which was found to consist mainly of astaxanthin.

Physiological conditions vary within and between species and the need for size and sex specific research is necessary to determine the rate of deposition of carotenoids in fish flesh. Based on our results, *Nyctiphanes australis* is a potential astaxanthin source for use in salmonid culture. Feeding studies are warranted to confirm the commercial use of this species as a pigmentation agent.

Addendum

The research in this paper was carried out by myself. Co-authors Bob Johannes and Peter Nichols initiated this research and played a supervisory role. Jock Young was responsible for collected of samples and reviewed this manuscript prior to publication. Mark Sands is acknowledged for his help with fluoride analysis.

Chapter 7. Summary and Conclusions

This study has consisted of a series of investigations on both *Euphausia superba* and *Nyctiphanes australis* in order to provide information on the life history, survival strategies and commercial potential of these species. A lipid based model was developed for *Euphausia superba* using the various individual studies that this thesis comprised. Lipids are fed into the model via food. Although lipids are used in short term starvation, krill lipid composition in the long term appears unconstrained by the quality and quantity of lipid of their feed. Krill appear to survive a harse regime of starvation but male krill cannot surviving mating. Male shunts all lipid reserves into reproduction and appears unable to recover. This may be a mechanism to decrease food resource competition during the winter. The conclusions of each of these components of model is outlined below with recomendations of futher studies.

Krill have long been recognised as the fat-rich food for whales. Energy storage in the form of lipid reserves is usually thought to be a prerequisite for survival in polar regions but Antarctic krill appear to be an exception to this rule. Lipids are not the primary source of metabolic energy for *Euphausia superba* during long periods of limited food supply. Instead, krill employ other mechanisms to withstand the seasonal pulse of nutrients. Krill can shrink up to a third of their body size when subjected to long period of starvation (>100 days). This decrease in body size allows krill to expend less energy while actively maintaining themselves in the water column. Additionally, both male and female krill regress sexually during starvation. This appears to be an effective mechanism because sexual maturity is of no relevance in the winter as sexual activity is restricted to the summer months.

In contrast, lipids are an energy source for krill during short term starvation. The digestive gland plays a dynamic role at the onset of starvation with the effects of starvation evident in this organ within days of limiting the food supply. Phospholipids were the major lipid class used during short term starvation, with levels of free fatty acid, polar lipid and cholesterol in the digestive gland providing indices of nutritional condition of *Euphausia superba* in the field. Sterols in the digestive gland indicate the recent dietary composition of krill. Algal sterols in the digestive gland can be used to quantify dietary input from individual phytoplanktonic species.

Lipids in the digestive gland can indicate recent dietary composition, the condition of krill, and can provide information on the food availability in the water column at the time of capture. This dietary information can assist in interpreting the physiological condition of the individual krill, and provides insight into the pattern of feeding in a swarm. Biomarkers are powerful tools when used in dietary studies. In order to maximise the effectiveness of biomarkers, additional research is required on characterising and quantifying biomarker compounds in Southern Ocean phytoplankton and other key planktonic organisms. Controlled feeding studies of krill using mixed phytoplankton diets are also necessary to further ground truth field based results using biomarkers.

Information on the biosynthetic capabilities of krill can help our understanding of how they survive periods of limited nutrients. Under laboratory conditions, krill were able to survive on a diet of *Phaeocystis pouchetii*. This abundant Antarctic phytoplanktonic species is deficient in essential fatty acids. This indicates that unlike other crustaceans, krill may possess the ability to convert short chain fatty acids to eicosapentaenoic and docosahexaenoic acids. These acids are essential for survival in crustaceans. Bacteria may also be a viable component of the krill diet. Bacterial cultures isolated from the stomach and digestive glands of krill were able to produce both the essential fatty acids; eicosapentaenoic and docosahexaenoic acids. Bacteria producing these necessary nutrients may be in a symbiotic relationship with krill. Alternatively, krill may be able to utilise a variety of nutrient sources during periods of low phytoplankton production, including bacteria.

To further to our understanding of krill energetics, the physiological cost of reproduction in male *Euphausia superba* was examined. Male reproductive costs in krill may exceed that of females. Despite male krill feeding actively during the reproductive period, levels of storage lipids (triacylglycerol) were depleted. Total lipid levels were substantially reduced in male krill during reproduction and this condition was followed by death. These observations may indicate a reproductive strategy whereby the reproductive life of males is reduced to fewer seasons than the female. The decrease in males in the Antarctic krill population reduces competition during the food-limited winter whereby resources are preferentially invested in females. Further investigations of

swarms in similar reproduction condition are warranted to substantiate this phenomenon.

Marine zooplankton tend to have higher lipid concentrations at higher latitudes. On average *Euphausia superba* had higher lipid levels than the temperate species, *Nyctiphanes australis*, but levels in *Euphausia superba* varied markedly with reproductive condition. Generally unsaturation levels increase with latitude as well, however, the degree of unsaturation of lipids in *Nyctiphanes australis* was considerably higher than those found in polar euphausiids. High dietary sources of polyunsaturated fatty acids or the ability to elongate and desaturate dietary fatty acids may be reasons for this. This indicates that differences in lipid levels and lipid composition between temperate and polar euphausiids may not be a function of temperature, but rather a function of the differences in food availability, life history and biochemical capabilities.

The biochemical composition of *Nyctiphanes australis* was investigated in terms of its possible use as an aquaculture feed. Being high in both polyunsaturated fatty acids and astaxanthin pigments meant that it would be a valuable supplement for farmed salmonids. High levels of fluoride in *Nyctiphanes australis* would not detract from its potential because salmon absorb fluoride in their skeleton only. Feeding studies using both *Euphausia*

References

- Ackman, R. G. and Eaton, C. A. 1966. Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters. III. Occurrence of eicosenoic and docosenic fatty acids in zooplankton *Meganyctiphanes norvegica* (M. Sars) and their effect on whale oil composition. Can. J. Biochem., 44, 1561-1566.
- Adelung, D., Buchholz, F., Culik, B. and Keck, A. 1987. Fluoride in tissues of krill *Euphausia superba* Dana and *Meganyctiphanes norvegica* M. Sars in relation to the moult cycle. Polar Biol., 7, 43-50.
- Al-Hasan, R. H., Ali, A. M. and Radwan, S. S. 1990. Lipids, and their constituent fatty acids, of *Phaeocystis* sp. from the Arabian Gulf. Mar. Biol., 105, 9-14.
- Anon. 1991. Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) Newsletter, 13, CCAMLR, Hobart.
- Anon. 1995. Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) Working Group-Krill Newsletter, January 1995, CCAMLR, Hobart.
- AOAC., (Association of Official Analytical Chemists). 1984. Official Methods of Analytical Chemists, 14th ed., Horwitz, W. (ed), Washington.
- Ashton, H. J., Farkvam, D. O. and March, B. E. 1993. Fatty acid composition of lipids in the eggs and alevins from wild and cultured chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci., 50, 648-655.
- Ashworth, T. K., Prasad, A. K. S. K. and Fryxell, G. A. 1990. AMERIEZ 88: Phytoplankton distributions across the Weddell Sea ice edge during austral winter. Eos, 71, 105.
- Ballantine, J. A., Lavis, A. and Morris, R. J. 1979. Sterols of the phytoplankton- effects of illumination and growth stage. Phytochem., 18, 1459-1466.
- Båmstedt, U. 1976. Studies on the deep-water pelagic community of Korsfjorden, western Norway: changes in the size and biochemical composition of *Meganyctiphanes norvegica* (Euphausiacea) in relation to its life cycle. Sarsia, 61, 15-30.

- Bargmann, H. E. 1937. The reproductive system of *Euphausia superba*. Discovery Reports, 23, 103-176.
- Barrett, S. M., Volkman, J. K., Dunstan, G. A. and LeRoi, J-M. 1995. Sterols of 14 species of marine diatoms (Bacillariophyta). J. Phycol., (in press).
- Bishop, D.G., Ritz, D.A., Hosie, G.W., Kenrick, J. R. and Olley, J. 1983. Fatty acid composition of the lipids of *Puffinus tenuirostris* (Temminck) in relation to its diet. J. Exp. Mar. Biol. Ecol., 71, 17-26.
- Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911-917.
- Bottino, N. R. (1975). Lipid composition of two species of Antarctic krill: *Euphausia superba* and *E. crystallorophias*. Comp. Biochem. Physiol., 50B, 479-484.
- Bourdier, G. G. and Amblard, C. A. 1989. Lipids in *Acanthodiaptomus denticornis* during starvation and fed on three different algae. J. Plankton Res., 11, 1201-1212.
- Braithwaite, R. W. and Lee, A. K. 1977. A mammalian example of semelparity. Amer. Natur., 111, 151-155.
- Brassell, S. C. and Eglinton, G. 1984. Lipid indicators of microbial activity in marine sediments. In Heterotrophic activity in the sea (eds. J. E. Hobbie and P. J. LeB. Williams), Academic Press London, pp. 105-136.
- Brown, M. R., Jeffrey, S. W. and Garland, C. D. 1989. Nutritional aspects of microalgae used in mariculture; a literature review. In: CSIRO Marine Laboratories Report, 205, Hobart, Tasmania.
- Budzinski, E., Bykowski, P., and Dutkiewicz, D. 1985. Possibilities of processing and marketing of products made from Antarctic krill. FAO Fisheries Technical Paper, 268, 1-46.
- Casalta, L. L., Olle, M., Blachere, A. and Ceccaldi, H. J. 1984. Sterols et fluor du krill antarctique, *Euphausia superba* Dana (Crustacea). Tethys, 11, 127-132.
- Castell, J. D., Lee, D. J. and Sinnhuber, R. O. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition. J. Nutr., 102, 93-100.
- Cheah, C. C. and Hansen, I. A. 1970. Wax esters in the stomach oil of petrels. Int. J. Biochem., 1, 198-202.

- Clarke, A. 1980. The biochemical composition of krill, *Euphausia superba* Dana, from South Georgia. J. Exp. Mar. Biol. Ecol., 43, 221-236.
- Clarke, A. 1983. Life in cold water: the physiological ecology of polar marine ectotherms. In Barnes, M., (editor). Oceanography and Marine Biology; an annual review, 21, 341-453.
- Clarke, A. 1984. Lipid content and composition of Antarctic krill, *Euphausia superba* Dana. Journal of Crustacean Biology, Special Edition, 4, 285-294.
- Clarke, A. 1987. A preliminary radioisotope study of the fate of ingested palmitic acid in the Antarctic krill, *Euphausia superba*. British Antarctic Survey Bulletin, 77, 99-107.
- Clarke, A. and Morris, D. J. 1983. Development of an energy budget for krill: The physiology and biochemistry of *Euphausia superba* Dana., Polar Biol., 2, 69-86.
- Clarke, A., and Wickins, J. F. 1980. Lipid content and composition of cultured *Penaeus merguensis* fed with animal food. Aquaculture, 20, 17-27.
- Claustre, H., Marty, J. C., Cassiani, L. and Dagaut, J. 1988/89. Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea: ecological implications. Marine Microbial Food Webs, 3, 51-66.
- Claustre, H., Poulet, S. A., Williams, R., Marty, J.-C., Coombs, S., Ben Mlih, F., Hapette, A. M. and Martin-Jezequel, V. 1990. A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. J. Mar. Biol. Ass. U.K., 70, 197-207.
- Cowey, C. B. and Sargent, J. R. 1977. Minireview. Lipid nutrition in fish. Comp. Biochem. Physiol., 57B, 269-273.
- Cowey, C. B. and Sargent, J. R. 1979. Nutrition. In: Fish Physiology. Vol. 8. Bioenergetics and Growth. Hoar, W. S., Randall, D. J. and Brett, J. R. eds., Academic Press, New York, pp. 1-69.
- D'Abramo, L. R. and S. Sheen. 1993. Polyunsaturated fatty acids in nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture, 115, 63-86.
- D'Abramo, L. R., Wright, J. S., Wright, K. H., Bordner, C. E. and Conklin, D. E. 1985. Sterol requirements of cultured juvenile crayfish, *Pacifastacus leniusulus*. Aquaculture, 49, 245-255.
- Dall, W. and Moriarty, D. J. W. 1983. Functional aspects of nutrition and digestion. In: Mantel L. H., (ed) The biology of Crustacea, vol. 5, Academic Press, New York, pp. 215-262.

- Dall, W., Chandumpai, A. and Smith, D. M. 1992. Fatty acid composition of organs and tissues of the tiger prawn *Penaeus esculentus* during the moulting cycle and during starvation. *Mar. Biol.*, 113, 45-55.
- Davidson, A. T. and Marchant, H. J. 1992a. The biology and ecology of *Phaeocystis* (Prymnesiophyceae). *Progress in Physiological Research*, 8, 1-45.
- Davidson, A. T. and Marchant, H. J. 1992b. Protist interactions and carbon dynamics of a *Phaeocystis* dominated bloom at an Antarctic coastal site. *Polar Biol.*, 12, 387-395.
- Denys, C. J., Poleck, T. P. and O'Leary, M. M. 1981. Biological studies of krill, austral summer 1979-80. *Ant. J. U.S.*, 15, 146-147.
- Dunkelblum, E., Tan, S. H., and Silk, P. J. 1985. Double-bond location in monounsaturated fatty acids by dimethyldisulfide derivatization and mass spectrometry: application to analysis of fatty acids in pheromone glands of four Lepidoptera. *J. Chem. Ecol.*, 11, 265-277.
- El-Sayed, Z. Z. and McWhinnie, M. A. 1979. Protein of the last frontier. *Oceanus*, 22, 13-20.
- Ellingsen, T. E. 1982. Biokjemiske studier over Antarktisk krill. Dr. Ing. Thesis, Univ. Trondheim, pp. 382.
- Ellingsen, T. E. and Mohr, V. 1981. Lipids in Antarctic krill. Composition and *post mortem* changes. *Proc. 11th. Scandinavian Symposium on Lipids* (Edited by Marcuse R.), SIK, Gothenborg, Sweden. pp. 110-116.
- Epifanio, C. E., Valenti, C. C. and Turk, C. L. 1981. A comparison of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* as food for the oyster, *Crassostrea virginica*. *Aquaculture*, 23, 347-353.
- Ettershank, G. 1983. Age structure of cyclical annual size change in the Antarctic krill, *Euphausia superba* Dana., *Polar Biol.* 2, 189-193.
- Everson, I. 1976. Antarctic krill: a reappraisal of its distribution. *Polar Records*. 18, 15-23.
- Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: Seasonal changes in body weight and the main biochemical composition of *Thysanoessa inermis* (Kroyer), *T. raschii* (M. Sars) and *Meganctiphanes norvegica* (M. Sars) in relation to environmental factors. *J. Exp. Mar. Biol. Ecol.*, 49, 103-120.
- Falk-Petersen, S. R., Gatten, R., Sargent, J.R. and Hopkins, C.C.E. 1981. Ecological investigations on the zooplankton community in

- Balsfjorden, northern Norway: seasonal changes in the lipid class composition of *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa raschii* (M. Sars) and *T. intermedius* (Krøyer). J. Exp. Mar. Biol. Ecol., 54, 209-224.
- Ferguson, C. F. and Raymont, J. K. B. 1974. Biochemical studies of marine zooplankton. XII. Further investigations on *Euphausia superba* Dana. J. mar. biol. Ass. U.K., 54, 719-715.
- Fessenden, R. J., and Fessenden, J. S. 1982. Organic chemistry, 2nd ed. Willard Grant Press, Boston Massachusetts.
- Foster, C. K. 1991. A survey of the quality of fishmeal available to the Tasmanian salmonid industry. In: Proceedings of the SALTAS research review seminar, CSIRO Marine Laboratories, Hobart, Tasmania, 165-175.
- Fraser, A. J. 1989. Triacylglycerol content as a condition index for fish, bivalve and crustacean larvae. Can. J. Fish. Aquat. Sci., 46, 1868-1873.
- Fricke, H., Gercken, G., Schreiber, W. and Oehlenschläger, J. 1984. Lipid, sterol and fatty acid composition of Antarctic krill (*Euphausia superba* Dana). Lipids, 19, 821-827.
- Fryxell, G. A. and Kendrick, G. A. 1988. Austral spring microalgae across the Weddell Sea ice edge: spatial relationships found along a northward transect during AMEREZ 83. Deep-Sea Res., 35, 1-20.
- Fujita, T., Satake, M., Watanabe T., Kitajima C., Miki W., Yamaguchi K. and Konosu S. 1983. Pigmentation of cultures of yellowtail with krill oil. Bull. Jpn. Sci. Soc. Fish., 66, 255-264
- Funk, V. A. and Hobson, L.A. 1991. Temporal variations in the carotenoid composition and content of *Euphausia pacifica* Hansen in Saanich Inlet, British Columbia. J. Exp. Mar. Biol. Ecol., 148, 93-104.
- Garrison, D. L. and Buck, K. R. 1989. The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsular regions. Polar Biol., 10, 211-219.
- Garrison, D. L., Buck, K. R. and Fryxell, G. A. 1987. Algal assemblages in the antarctic pack-ice plankton. J. Phycol., 23, 564-572.
- Giese, A. G. 1966. Lipids in the economy of marine invertebrates., Physiol. Rev., 46, 244-298.
- Goodwin, T. W. 1984. The biochemistry of the Carotenoids, Vol. II. Animals, 2nd edition. Chapman and Hall, London, pp. 224.

- Grantham, G. J. 1977. The Utilization of krill, Southern Ocean Fisheries Survey Program, FAO, pp. 3-16.
- Grave, H. 1981. Fluoride content of salmonids fed on Antarctic krill. *Aquaculture*, 24, 191-196.
- Guckert, J. B., Antworth, C. P., Nichols, P. D. and White, D. C. 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol. Ecol.*, 31, 147-158
- Gillan, F. T., McFadden, G. I., Wetherbee, R. and Johns, R. B. 1981. Sterols and fatty acids of an Antarctic sea ice diatom *Stauroneis amphioxys*. *Phytochem.*, 20, 1935.
- Guillard, R. R. L. and Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.*, 8, 229-239.
- Hagen, W. 1988. Zur Bedeutung der Lipide im antarktischen Zooplankton [On the significance of lipids in Antarctic zooplankton.] Alfred-Wegener-Institut für Polar- und Meeresforschung. *Berichte zur Polarforschung*, 49, 1-129.
- Hairton, N. J. Jr. 1979. The adaptive significance of color polymorphism in two species of *Diaptomus* (Copepoda). *Limnol. Oceanogr.* 24, 15-37.
- Harrington, S. A. and Ikeda, T. 1986. Laboratory observations on spawning, brood size and egg hatchability of the Antarctic krill *Euphausia superba* from Prydz Bay, Antarctica. *Mar. Biol.*, 92, 231-235.
- Harris, G. P., Nilsson, C., Clementson, L. and Thomas, D. 1987. The water masses of the east coast of Tasmania: seasonal and interannual variability and the influence of phytoplankton biomass and productivity. *Aust. J. Mar. Freshw. Res.*, 38, 569-590.
- Heath, J. R. and Barnes, H. 1970. Some changes in biochemical composition with season and during the moulting cycle of the common shore crab *Carcinus maenas* (L). *J. Exp. Mar. Biol. Prog. Ser.*, 26, 199-233.
- Hewes, C. D., Holm -Hansen, O. and Sakshaug, E. 1985. Alternate carbon pathways at lower trophic levels in the Antarctic food web. In: Antarctic nutrient cycles and food webs, Siegfried, W. R., Condy P. R., and Laws, R. M. Berlin, eds. Heidelberg, Springer Verlag, pp. 227-283.
- Higginbottom, I. R. and Hosie, G. W. 1989. Biomass and population structure of a large aggregation of krill near Prydz Bay, Antarctica., *Mar. Ecol. Prog. Ser.* 58, 197-203.

- Holm -Hansen, O. 1985. Nutrient cycles in Antarctic marine ecosystems. In: Antarctic nutrient cycles and food webs, Siegfried, W. R., Condy P. R., and Laws, R. M. Berlin, eds. Heidelberg, Springer Verlag, pp. 6-10.
- Hosie, G. W., Ikeda, T. and Stolp, M. 1988. Distribution, abundance and population structure of the Antarctic krill (*Euphausia superba* Dana) in the Prydz Bay region, Antarctica., Polar Biol. 8, 213-224.
- Ikeda, T. 1985. Life history of Antarctic krill *Euphausia superba*: A new look from an experimental approach. Bull. Mar. Sci., 37, 599-608.
- Ikeda, T. and Dixon, P. 1982. Body shrinkage as a possible over-wintering mechanism of the Antarctic krill, *Euphausia superba* Dana. J. Exp. Mar. Biol. Ecol., 2, 143-151.
- Ikeda, T. and Thomas, P. G. 1987. Moulting interval and growth of juvenile Antarctic krill (*Euphausia superba*) fed different concentrations of the diatom *Phaeodactylum tricornutum* in the laboratory. Polar Biol., 7, 339-343.
- Iverson, S. J. 1993. Milk secretion in marine mammals in relation to foraging: can milk fatty acids predict diet? Symp. Zool. Soc. Lond., 66, 263-291.
- Kanazawa, A. 1985. Nutrition of penaeid prawns and shrimps. In: *Proceedings of the First International Conference on the Culture of Penaeid Prawn/Shrimp*, Iloilo City, The Philippines, 1984. SEAFDEC Aquaculture Department, 123-130.
- Kanazawa, A., Teshima, S. I. and Ono, K. M. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp. Biochem. Physiol., 63B, 295-298.
- Kanazawa, A., Teshima, S. I., Sakamoto, M. and Awal, M. A. 1980. Requirement of *Tilapia zilli* for essential fatty acids. Bull. Jap. Soc. Sci. Fish., 46, 1353-1356.
- Kanazawa, A., Teshima, S., Endo, M. and Kayama, M. 1978. Effects of eicosapentaenoic acid on growth and fatty acid composition of prawn, *Penaeus japonicus*. Mem. Fac. Fish. Kagoshima Uni., 28, 21-26.
- Karl, D. M. 1993. Microbial processes in the Southern Oceans. In Antarctic Microbial Processes, Friedmann, E. I., ed. Wiley-Liss, Inc. New York, pp. 1-63.
- Kawaguchi, K. S., Ishikawa, S. and Matsuda, O. 1986. The overwintering strategy of Antarctic krill, (*Euphausia superba* Dana) under the coastal fast ice off the Ongul Island in Lutzow-Holm Bay, Antarctica. Mem. natl. Inst. Polar Res., Spec. Issue 44, 67-85.

- Kirkwood, J. M. 1982. A guide to the Euphausiacea of the Southern Ocean. Australian National Antarctic Research Expeditions. ANARE research notes, 1, pp. 1-45.
- Klindt, H. and Zwack, F. 1984. A method for acoustic estimation of krill (*Euphausia superba* Dana) abundance applied to FIBEX data. Arch. FischWiss., 34, 121-144.
- Lall, S. P. 1991. Salmonid nutrition and feed production. In: Cook, R. H and Pennell, W. (eds.) Proceedings of the special session on salmonid aquaculture, World Aquaculture Society, feb. 16, 1989. Los Angeles, USA. Can. Tech. Rep. Fish. Aq. Sci., 1831, pp. 107-123.
- Lancelot, C., Mathot, S., Becquevort, S., Dandois, J.-M. and Billen, G. 1993. Carbon and nitrogen cycling through the microbial network of the marginal ice zone of the Southern Ocean with particular emphasis on the northwestern Weddell Sea. In Plankton Ecology and Marine Biogeochemistry, Caschetto, S., ed. Belgian Science Policy Office, 1, pp. 1-102.
- Latogurskij, V. J. 1973. Besonderheiten in der Biologie des Krills aus dem Bereich von Suedgeorgien waehrend der Sommer- und Wintersaison 1969-1970 (russ.). Transl. from Trudy Atlant Nitro. 53, 135-150.
- Laureillard, J. and Saliot, A. 1993. Biomarkers in organic matter produced in estuaries: a case study of the Krka estuary (Adriatic Sea) using the sterol marker series. Mar. Chem., 43, 247-261.
- Lee, R. F., Nevenzel, J. C., Paffenhofer, G. A. and Benson, A. A. 1970. The metabolism of wax esters and other lipids by the marine copepod, *Calanus helgolandicus*. J. Lipid Res., 11, 237-240.
- Lehninger, A. L. 1975. Biochemistry, 2nd ed. Worth Publishers Inc., New York. NY. pp. 1104.
- Leray, C., Nanotte, g., Rouband, P. and Leger, C. 1985. Incidence of n-3 essential fatty acid deficiency on trout reproductive processes. Reprod. Nutr. Dev., 25, 567-581.
- Lewis, T. L., Stumpers, A. C. and Davies, D. M. 1987. Analysis of fluoride in foodstuffs and krill. Proceedings of the 9th Australian Symposium of Analytical Chemists, pp. 168-171.
- Lin, H., Romsos, D. R., Tack, P. I. and Leveille, G. A. 1977. Influence of dietary lipid on lipogenic enzyme activities in coho salmon (*Oncorhynchus kisutch* (Waobaum)). J. Nutr., 107, 846-854.
- Littlepage, J. 1964. Seasonal variation in lipid content on two Antarctic marine Crustacean. In: Biologie antarctique R. Carrick, M. W.

- Holdgate and J. Prevost (Editors), 1er Symp. SCAR, Paris 2.-8.9. 1962, Hermann, Paris, pp. 463-470.
- Loeblich, A. R. III, Smith, V. E. 1968. Chloroplast pigments of the marine dinoflagellate *Gymnodinium resplendens*. *Lipids*, 3, 3-15.
- Lutter, S., Taasen, J. P., Hopkins, C. C. E. and Smetacek, V. 1989. Phytoplankton dynamics and sedimentation processes during spring and summer in Balsfjord, Northern Norway. *Polar Biol.*, 10, 113-124.
- Mackie, P. R., Whittle, K. J. and Hardy, R. 1974. Hydrocarbons in the marine environment. I. n-alkanes in the Firth of Clyde. *Est. Coastl. Mar. Sci.*, 6, 301-313.
- Mackintosh, N. A. 1967. Maintenance of living *Euphausia superba* and frequency of moults. *Nor. Hvalfangst-Tid.*, 56, 97-102.
- Makarov, R. R. 1976. Reproduction of *Euphausia superba* (Dana) (Crustacean: Euphausiacea). *Trudy vses. nauchno-issled. Inst. morsk. ryb. Khoz. Okeanogr*, 110, 85-89.
- Maoka, T., Katsuyama, M., Kaneko, N. and Matsuno, T. 1985. Stereochemical investigations of carotenoids in the Antarctic krill *Euphausia superba*. *Bull. Jpn. Sci. Soc. Fish.*, 51, 1671-1673.
- Marchant, H. J. and Nash, G. V. 1986. Electron microscopy of gut contents and faeces of *Euphausia superba* Dana. *Memoirs National Institute of Polar Research, Special Issue*, 40, 167-177.
- Marr, J. 1962. The natural history and geography of the Antarctic krill (*Euphausia superba* Dana). *Discovery Report*, 32, 33-464.
- Mauchline, J. 1960. The biology of the euphausiid crustacean *Meganyctiphanes norvegica* (M. Sars). *Proc. R. Soc. Edinb. B (Biol.)*, 67, 141-179.
- Mauchline, J. 1960. The biology of *Thysanoessa raschi* (M. Sars) with a comparison of its diet with that of *Meganyctiphanes norvegica* (M. Sars). In: *Some Contemporary Studies in Marine Science*. H. Barnes (Editor), London, Allen and Unwin, pp. 493-510.
- Mauchline, J. 1980. The biology of mysids and euphausiids. *Adv. Mar. Biol.*, 18, pp. 1-677.
- Mauchline, J. and Fisher, L. R. 1969. The biology of euphausiids., *Adv. Mar. Biol.*, 7, 1-454.
- Mayzaud, P., Eaton, C. A. and Ackman, R. G., 1976. The occurrence and distribution of octadecapentanoic acid in a natural plankton population. A possible food chain index. *Lipids*, 11, 858-862.

- Miki, W., Yamaguchi, K., Konosu, S., Takane, T., Satake, M., Fujita, T., Kuwabara, H., Shimeno, S. and Takeda, M. 1985. Origin of tunaxanthin in the integument of yellowtail (*Seriola quinqueradiata*). *Comp. Biochem. Physiol.*, 80B, 195-201.
- Miller, D. G. M. 1991. Exploitation of Antarctic marine living resources: a brief history and a possible approach to managing the krill fishery. *S. Afr. J. mar. Sci.*, 10, 321-339.
- Miller, D. G. M. and Hampton, I. 1989. Biology and ecology of the Antarctic krill (*Euphausia superba* Dana): a review. *Biomass*, 9, 1-166.
- Morris, D. J. and Watkin, J. 1986. Krill swarms: Does proximity imply similarity? *BIOMASS Newsletter*, 8, 6-7.
- Morris, R. J. and Sargent, J. R. 1973. Studies on the lipid metabolism of some oceanic crustaceans. *Mar. Biol.*, 22, 77-83.
- Nemoto, T. 1986. Chlorophyll pigments in the stomachs of euphausiids. *J. Oceanogr. Soc. Jap.*, 17, 50-55.
- New, M. B. 1976. A review of the dietary studies with shrimp and prawns. *Aquaculture*, 9, 101-144.
- Nichols, D. S., Nichols, P. D. and McMeekin, T. A. 1993. Polyunsaturated fatty acids in Antarctic bacteria. *Antarctic Sci.*, 5, 149-160.
- Nichols, D. S., Nichols, P. D. and Sullivan, C. W. 1993. Seasonal studies of the fatty acid, sterol and hydrocarbon composition of Antarctic sea-ice diatom communities collected during the spring bloom of 1989 in McMurdo Sound. *Antarctic Sci.*, 5, 271-279.
- Nichols, P. D., Guckert, J. B., and White, D. C. 1986b. Determination of monounsaturated fatty acid double bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. *J. Microbiol. Methods*. 5, 49-55.
- Nichols, P. D., Jones, G. J., de Leeuw, J. W. and Johns, R. B. 1984. The fatty acid sterol composition of two marine dinoflagellates. *Phytochem.*, 23(5), 1043-1047.
- Nichols, P. D., Palmisano, A. C., Rayner, M. S. Smith, G. A. and White, D. C. 1990. Occurrence of novel C₃₀ sterols in Antarctic sea-ice communities during a spring bloom. *Organic Geochem.*, 15, 503-508.
- Nichols, P. D., Palmisano, A. C., Rayner, M. S., Smith, G. A. and White D. C. 1989. Changes in the lipid composition of Antarctic sea-ice diatom communities during a spring bloom: an indication of community physiological status. *Antarctic Sci.*, 1, 133-140.

- Nichols, P. D., Palmisano, A. C., Smith, G. A. and White, D. C. 1986a. Lipids of the Antarctica sea ice diatom *Nitzschia cylindrus*. *Phytochem.*, 25, 1649-1653.
- Nichols, P. D., Skerratt, J. H., Davidson, A., Burton, H. and McMeekin, T. A. 1991. Lipids of cultured *Phaeocystis pouchetii*: signatures for food-web, biogeochemical and environmental studies in Antarctica and the Southern Ocean. *Phytochem.*, 30, 3209-3214.
- Nicol, S. 1987. Some limitations on the use of the lipofuscin ageing technique. *Mar. Biol.*, 93, 609-614.
- Nicol, S. 1989. Apparent independence of the spawning and moulting cycles in Antarctic krill (*Euphausia superba* Dana). *Polar Biol.*, 9, 371-375.
- Nicol, S. 1989. Who's counting on krill? *New Scient.*, 124 (1690), 38-41.
- Nicol, S. 1990. The Age-old problem of krill longevity. *BioSci.*, 40, 833-36.
- Nicol, S. and Stolp, M. 1989. Sinking rates of cast exoskeletons of Antarctic krill (*Euphausia superba* Dana) and their role in the vertical flux of particulate matter and fluoride in the Southern Ocean. *Deep-Sea Res.*, 36, 1753-1762.
- Nicol, S. and Stolp, M. 1991. Moulting, feeding and fluoride concentration of the Antarctic krill *Euphausia superba* Dana. *J. Crustacean Biol.*, 11, 10-16.
- Nicol, S., Stolp, M., Cochran, T., Geijssels, P. and Marshall, J. 1992. Summer growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean sector of the Southern Ocean during summer. *Mar. Ecol. Prog. Ser.*, 89, 175-181.
- Nicol, S., Stolp, M. and Nordstrom, O. 1992. Changes in the gross biochemical and mineral content accompanying the moult cycle in the Antarctic krill *Euphausia superba*. *Mar. Biol.*, 113, 201-209.
- O'Brien, D. P. 1988. Surface schooling behaviour of the coastal krill *Nyctiphanes australis* (Crustacea: Euphausiacea) off Tasmania, Australia. *Mar. Ecol. Prog. Ser.*, 12, 219.
- Odate, K. 1979. An euphausiid crustacea exploited along the Sanriku and Joban coast. *Bulletin Tohoku Regional Fisheries Research Laboratory* 40 (January), pp. 15-25.
- Oehlenschläger, J. and Mantney, M. 1982. Fluoride content of antarctic marine animals caught off Elephant Island. *Polar Biol.*, 1, 125-127.
- Partridge, L. and Farquhar, M. 1981. Sexual activity reduces lifespan of male fruitflies., *Nature*, 294, 580-582.

- Patterson, G. W. 1987. Sterol synthesis and distribution in Algal Phylogeny. In: The metabolism, structure and Function of Plant Lipids (eds. P. K. Stumpf, J. B. Mudd, W. D. Nes), Plenum Press, New York., pp. 631-636.
- Pierce, R. W., Van der Veen, J. and Olcott, H. S. 1969. Proximate and lipid analysis of krill (*Euphausia* species) and Red crab (*Pleuroncodes planipes*). J. Agricult. Food Chem., 17, 367-369.
- Pieters, H., Kluytmans, J. H., Zandee, D. I. and Cadée, G. C. 1980. Tissue composition and reproduction of *Mytilus edulis* in relation to food availability. Netherlands J. Sea Res., 14, 349-361.
- Pike, I. H., Andorsdottir, G. and Mundheine, H. 1990. The role of fishmeal in diets for salmonids. IAFMN, No. 24, March, 1990, pp. 34.
- Poleck, T. P. and Denys, C. J. 1982. Effects of temperature on the moulting, growth and maturation of the Antarctic krill *Euphausia superba* (Crustacea: Euphausiacea) under laboratory conditions. Mar. Biol., 70, 255-265.
- Ponat, A. and Adelung, D. 1983. Studies to establish an optimal diet for *Carcinus maenas*. 3. Vitamin and quantitative lipid requirements. Mar. Biol., 74, 275-279.
- Quetin, L. B. and Ross, R. M. 1984. School composition of the Antarctic krill *Euphausia superba* in the waters of the Antarctic peninsula in the austral summer of 1992. J. Crust. Biol., 4, 96-106.
- Quetin, L. B. and Ross, R. M. 1991. Behavioural and Physiological Characteristics of the Antarctic Krill, *Euphausia superba*. Amer. Zool., 31, 49-63.
- Quetin, L. B., Ross, R. M. and Clarke, A. 1994. Krill energetics: seasonal and environmental aspects of the physiology of *Euphausia superba*. Southern Ocean Ecology the BIOMASS Prospective. S. Z., El-Sayed, ed. Cambridge University Press, Cambridge, England, 165-184.
- Rakusa-Suszczewski, S. and Zdanowski, M. K. 1989. Bacteria in krill (*Euphausia superba* Dana) stomach. Acta Protozoologica, 28, 87-90.
- Raymont, J. E. G., Stinivasagam, R. T. and Raymont, J. K. B. 1971. Biochemical studies of marine zooplankton. IX. The biochemical composition of *Euphausia superba*. J. Mar. Biol. Ass. U.K., 51, 581-588.
- Reinhardt, S. B. and Van Vleet, E. S. 1986. Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Mar. Biol., 91, 149-159.

- Ritz, D. A. and Hosie, G. 1982. Production of the euphausiid *Nyctiphanes australis* in Storm Bay, South-Eastern Tasmania. *Mar. Biol.*, 68, 103-108.
- Rogers, S. I. and Lockwood, S. J. 1990. Observations of coastal fish fauna during a spring bloom of *Phaeocystis pouchetii*: (Hariot) in the eastern Irish Sea. *J. Mar. Biol. Ass. U.K.*, 70, 249-253.
- Ross, R. M. and Quetin, L. B. 1983. Spawning frequency and fecundity of the Antarctic krill *Euphausia superba*., *Mar. Biol.*, 77, 201-205.
- Ross, R. M. and Quetin, L. B. 1986. How productive are Antarctic krill? *Bioscience*. 36, 264-269.
- Ross, R. M. and Quetin, L. B. 1988. *Euphausia superba*: a critical review of estimates of annual production. *Comp. Biochem. Physiol.*, 90B (3), 499-505.
- Saether, O., Ellingsen, T. E. and Mohr, V. 1985. The distribution of lipid in the tissues of Antarctic krill, *Euphausia superba*. *Comp. Biochem. Physiol.*, 81, 609-614.
- Saether, O., Ellingsen, T. E. and V. Mohr. 1986. Lipids of North Atlantic krill. *J. Lipid Res.*, 27, 274-285.
- Sargent, J. R. 1976. Structure, metabolism and function of lipids in marine organisms. In: *Biochemical and Biophysical Perspectives in Marine Biology*. D. C. Malins and J. R. Sargent (Editors), Academic Press, London, 3, pp. 149-212.
- Sargent, J. R. and Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis* during mid-winter. *Mar. Biol.*, 62, 131-137.
- Sargent, J. R. and Henderson, R. L. 1987. Marine (n-3) polyunsaturated fatty acids. In *Developments in oils and fats* (ed. R. J. Hamilton), Elsevier, Amsterdam.
- Sargent, J. R. and Whittle, K. J. 1981. Lipids and hydrocarbons in the marine food web. In: *Analysis of marine ecosystems*. A. R. Longhurst (Editor), London, Academic Press, pp. 491-533.
- Sargent, J. R., Eilertsen, H. C., Falk-Petersen, S. and Taasen, J. P. 1985. Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. *Mar. Biol.*, 85, 109-116.
- Sargent, J. R., Eilertsen, H. C., Falk-Petersen, S. and Taasen, J. P. 1985. Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. *Mar. Biol.*, 85, 109-116.

- SAS/Statistical Guide for Personal Computers, Users Guide, Release 6.03
Edition SAS/STAT™ Institute Inc., Cary, NC, USA, 1988.
- Scott, A. P. and Middleton, C. 1979. Unicellular algal diet as a food for turbot (*Scophthalmus maximus* L.) larvae; the importance of longchain polyunsaturated fatty acids. *Aquaculture*, 18, 227-240.
- Sheard, K. (1953). Taxonomy, distribution and development of Euphausiacea (Crustacea). Rep. B.A.N.Z. antarct. Res. Exped., Ser. B, Vol. 8, pp. 1-72.
- Shigeru, A., Tetsu, M., Wataru, M., Katsumi, Y., Shoji, K., Satake, M. and Takao, F. 1987. Pigmentation of juvenile coho salmon with carotenoid oil extracted from antarctic krill. *Aquaculture*, 66, 255-264.
- Shimizu, C., Ibrahim, A., Tokoro, T. and Shirakawa, Y. 1990. Feeding stimulation in sea bream, *Pagrus major*, fed diets supplemented with Antarctic krill meals. *Aquaculture*, 89, 43-53.
- Shuman F., R. and Lorenzen C., J. 1975. Quantitative degradation of chlorophyll by a marine herbivore. *Limnol. Oceanogr.*, 20(4), 580-586.
- Sieburth, J. McN. 1960. Acrylic acid, an "antibiotic" principle in *Phaeocystis pouchetii* in Antarctic waters. *Science*, N.Y., 132, 676-667.
- Siegel, V. 1987. Age and growth of Antarctic *Euphausiacea* (Crustacea) under natural conditions. *Mar. Biol.*, 96, 483-495.
- Sikes, E. L. and Volkman, J. K. 1993. Using alkenone unsaturation ratios to estimate past sea surface temperatures (SST) in the Southern Ocean. Abstracts of the American Geological Society, pp. 445.
- Skira, I. J. 1986. Food of the Short-tailed Shearwater, *Puffinus tenuirostris*, in Tasmania. *Aust. Wildl. Res.*, 13, 481-488.
- Sloan, N. A. and Fulton, J. 1987. 1. Plankton- Euphausiids (trawl fishery). In: Harbo, R. M. and Jamieson, G. S. (eds.) Status of Invertebrate Fisheries off the Pacific Coast of Canada (1985/86). Canadian Technical Report of Fisheries and Aquatic Sciences, 1576, pp. 9-15.
- Smith, A. E. and Morris, I. 1980. Synthesis of lipid during photosynthesis by phytoplankton of the Southern Ocean. *Science*, Wash., D.C., 207, 197-199.
- Spiridonov, V. A., Gruzov, E. N. and Pushkin, A. F. 1985. Observations on schools of the Antarctic *Euphausia superba* (Crustacea, Euphausiacea) under ice. (In Russian). *Zool. Zh.*, 64, 1655-1660.
- Storebakken, T. 1988. Krill as a potential feed source for salmonids. *Aquaculture*, 70, 193-205.

- Storebakken, T. and No, H. K. 1992. Pigmentation of rainbow trout. *Aquaculture*, 100, 209-229.
- Strain H. H., Svec W. A., Aizetmuller K., Grandolfo M. C., Katz T. T., Kjosen H., Norgard S., Liaaen-Jensen S., Haxo F. T., Wegafahrt P. and Rapoport H. 1971. The structure of peridinin, the characteristic dinoflagellate carotenoid. *J. Amer. Chem. Soc.*, 93, 1823-1824.
- Stryer L. 1988. *Biochemistry*, (3rd ed), Stanford Uni. W.H. Freeman and Co. New York.
- Suzuki, T. and Shibata, N. 1990. The utilization of Antarctic krill for human food. *Food Rev. Int.*, 6(1), 119-147.
- Szewielow, A. 1981. Fluoride in Antarctic krill (*Euphausia superba* Dana). *Meeresforschung*, 28, 244-246.
- Takeuchi, T. and Watanabe, T. 1977. Requirements of the carp for essential fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries*, 43, 541-551.
- Takeuchi, T., Watanabe, T. and Ogino, C. 1978. Supplementary effect of lipids in a high protein diet of rainbow trout. *Bull. Jap. Soc. Scient. Fish.*, 44, 677-681.
- Tande, K. S. and Båmstedt, U. 1987. On the trophic fate of *Phaeocystis pouchetii*. I. Copepod feeding rates on solitary cells and colonies of *P. pouchetii*. *Sarsia*, 72, 313-320.
- Tanoue, E. and Hara, S. 1986. Ecological implications of fecal pellets produced by the Antarctic krill *Euphausia superba* in the Antarctic Ocean. *Mar. Biol.*, 91, 359-369.
- Teshima, S. 1982. Sterol metabolism. *Proceedings of the Second Conference on Aquaculture Nutrition; Biochemical and Physiological Approaches to Shellfish Nutrition*. G. D. Pruder, C. J. Langdon and D. E. Conklin, eds. Louisiana State University Press, Baton Rouge, 205-215.
- Teshima, S. and Kanazawa, A. 1971. Biosynthesis of sterols in the lobster, *Panulirus japonica*, the prawn, *Penaeus japonicus*, and the crab, *Portunus trituberculatus*. *Comparative Biochemistry and Physiology*, 38B, 597-602.
- Teshima, S. and Kanazawa, A. 1980. Transport of dietary lipids and role of serum lipoproteins in the prawn. *Bull. Jap. Soc. Scient. Fish.*, 46, 51-55.

- Thomas, P. G. and Ikeda, T. 1987. Sexual regression, shrinkage, rematuration and growth of spent female *Euphausia superba* in the laboratory. *Mar. Biol.*, 95, 357-363.
- Trivers, R. L. 1972. Sexual Selection and the Decent of Man 1871-1971, B. Campbell (Editor), Aldine, Chicago, pp. 136-179.
- Van Voorhies, W. A. 1992. Production of sperm reduces nematode lifespan., *Nature*, 360, 456-458.
- Verity, P. G. and Smayda, T. J. 1989. Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepoda): ingestion, egg production, and growth of nauplii. *Mar. Biol.*, 100, 161-171.
- Virtue, P., Nichols, P. D., Nicol, S., McMinn, A. and Sikes, E. L. 1993a. The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. *Antarctic Sci.*, 5(2), 169-177.
- Virtue, P., Nicol, S. and Nichols, P. D. 1993b. Changes in the digestive gland of *Euphausia superba* during short-term starvation: lipid class, fatty acid and sterol content and composition. *Mar. Biol.*, 117, 441-448.
- Virtue, P. Nichols, P. D. and Nicol, S. 1995. Dietary related mechanisms of survival in *Euphausia superba*: biochemical changes during long term starvation and bacteria as a possible source of nutrition. Proceeding from the SCAR VI Biological Symposium. B. Battaglia, J. Valencia, D.W.H. Walton (Editors). Cambridge University Press, (in press).
- Volkman, J. K. 1986. A review of sterol markers for marine and terrigenous organic matter. *Organic Geochem.*, 9, 83-99.
- Volkman, J. K. and Nichols P. D. 1991. Applications of thin layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. *J. Planer Chromatogr.*, 4, 19-26.
- Volkman, J. K., Everitt, D. A. and Allen, D. I. 1986. Some analysis of lipid classes in marine organisms, sediments and seawater using thin-layer chromatography- flame ionisation detection. *J. Chromatogr.*, 6, 147-162.
- Volkman, J. K., Jeffrey, S. W., Nichols P. D., Rogers, G. I. and Garland, C. D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.*, 128, 219-240.

- Walford, J. and Lam, T. J. 1987. Effect of feeding with micro-capsules on the content of essential fatty acids in live foods for larvae of marine fishes. *Aquaculture*, 61, 219-229.
- Watanabe, T. 1982. Lipid nutrition in fish. *Comp. Biochem. Physiol.*, 73B, 3-15.
- Watanabe, T., Kitajima, C. and Fujita, S. 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, 34, 115-143.
- Watkins, J. L. 1986. Variation in the size of Antarctic krill, *Euphausia superba* Dana, in small swarms. *Mar. Ecol. Prog. Ser.*, 31, 67-73.
- Webb, B. F. 1976. Aspects of the biology of jack mackerel, *Trachurus declivis* (Jenyns), from south east Australian waters. *Tasmanian Fisheries Research*, 10(1), 1-17.
- Weisse, T. 1983. Feeding of calanoid copepods in relation to *Phaeocystis pouchetii* blooms in the German Wadden Sea off Sylt. *Mar. Biol.*, 74, 87-94.
- White, D. C. 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. In *Microbes in their natural environments* (eds. J. H. Slater et al.), Cambridge University Press. pp. 33-66.
- White, D. C., Davis, W. M., Nickels, J. S., King, J. D. and Bobbie, R. J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia*, 40, 51-62.
- Wiborg, K. F. 1971. Investigation on euphausiids in some fjords on the west coast of Norway in 1966-1969. *FiskDir. Skr. ser. HavUnders.*, 16, 10-35.
- Williams, H., Pullen, G., Kucerans, G. and Waterworth, C. 1987. The jack mackerel purse seine fishery in Tasmania, 1986-1987. *Tech. Rep. Deep. Sea Fish. Tasm.*, 19, pp. 1-32.
- Williams, R., Kirkwood, J. M. and O'Sullivan, D. 1983, FIBEX cruise zooplankton data. Australian National Antarctic Research Expeditions. ANARE research notes, 7, pp. 1-165.
- Wright S. W. and Jeffrey S. W. 1987. Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. *Mar Ecol. Prog. Ser.*, 38, 259-266.
- Wright, S. W., Jeffrey, S. W., Mantoura, R. F. C., Llewellyn, T., Bjornland, T., Repeta, D. and Welschmeyer, N. 1991. Improved HPLC method for

- the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser., 77, 183-196.
- Yamaguchi, Y. and Shibata, Y. 1982. Standing stock and distribution of phytoplankton chlorophyll in the Southern Ocean south of Australia. Transactions of the Tokyo University of Fisheries, 5, 111-128.
- Yingst, W. L., III, and Stickney, R. R. 1980. Growth of caged channel catfish fingerlings reared on diets containing various lipids. Progressive Fish-Culturists, 42, 24-26.
- Yongmanitchai, W. and Ward, P. O. 1991. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Env. Microbiol., 57, 419-425.
- Young, J. W., Jordan, A. R., Bobbi, C., Johannes, R. E., Haskard, K. and Pullen, G. 1993. Seasonal and interannual variations in krill (*Nyctiphanes australis*) stocks and their relationship to the fishery for jack mackerel (*Trachurus declivis*) off eastern Tasmania, Australia. Mar. Biol., 116, 9-18.
- Zobell, C. E. 1946. Marine Microbiology, Waltham: Chronica Botanica, pp. 240.

Appendix 1. Sexual maturity stages of *Euphausia superba*

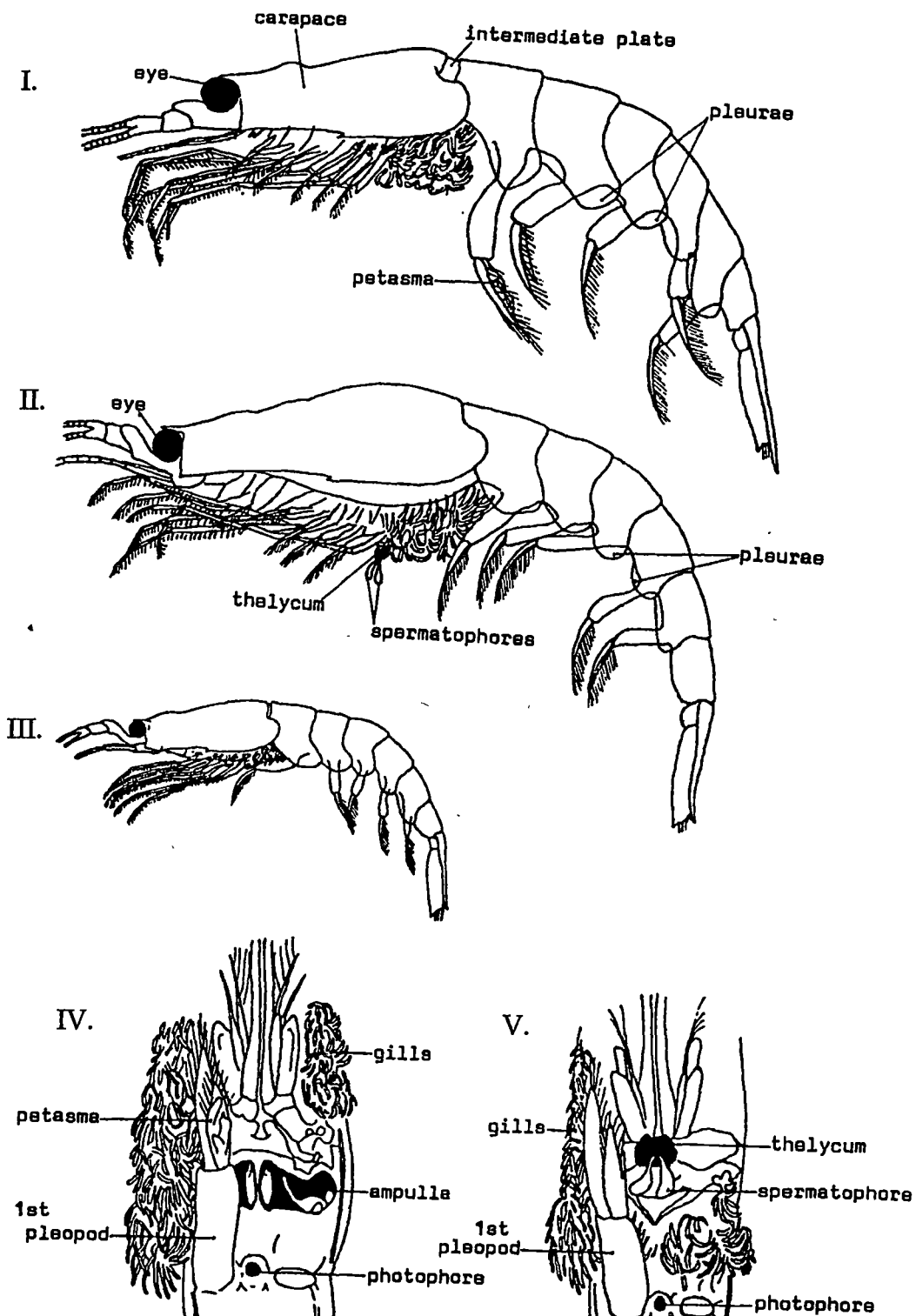
(extert from Krikwood (1982), after Makarov and Denys (1980)).

1. **Juvenile:** External sexual features (i.e. petasmas and ampullae in males, thelycum in females) not present. Individuals may be sexed by dissection, but as they are not in or approaching reproductive condition this is generally considered unnecessary.
2. **Sub-Adult:**
Male: Developing petasmas visible. Ampullae small and pale.
2AM: Petasmas single undivided lobes (b).
2BM: Petasmas bilobed, but have no wing (c).
2CM: Petasmas almost fully developed, with "wings" present (d).
Female: Developing thelycum visible, its colour ranging from white to pale red.
3. **Adult:**
Male: (I): Petasmas fully developed (e). Ampullae usually bright red and clearly visible beneath the gills (IV).
3AM: Spermatophores not present in ampullae
3BM: Spermatophores extruding from pore on ampullae, or present within and easily ejected by pressure on the ampullae.

Female: (II): Thelycum bright red, fully developed, and clearly visible through the gills (V).
3AF: Thelycum bears no spermatophores.
3BF: Spermatophores attached to thelycum. Empty space between ovary and body wall.
3CF: Spermatophores attached. Ovary fills thoracic space.
3DF: Spermatophores attached. Carapace noticeably swollen by enlarged ovary. (II).
3EF: Carapace swollen in contour, but with large hollow space owing to the recent spawning of eggs.

This system is basically the same as that proposed by Mauchline et. al. (1979). The only changes are in the division of sub-adult males into 2AM, 2BM and 2CM and the addition of a new stage (3EF) to denote post-spawn females. The numeration system has also been modified.

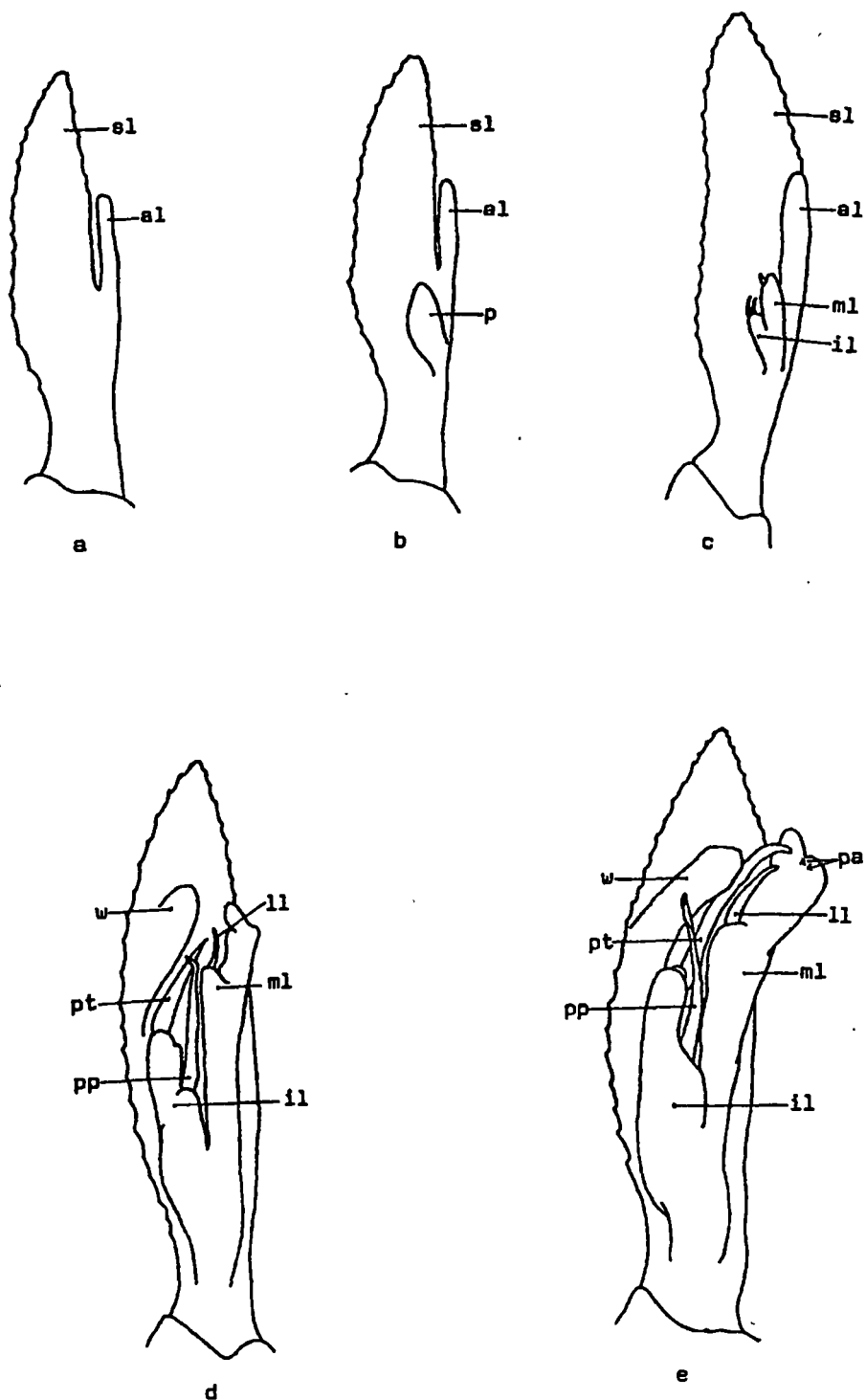
Appendix 1. (I-V)



Reproduction and sexual maturation of *Euphausia superba*

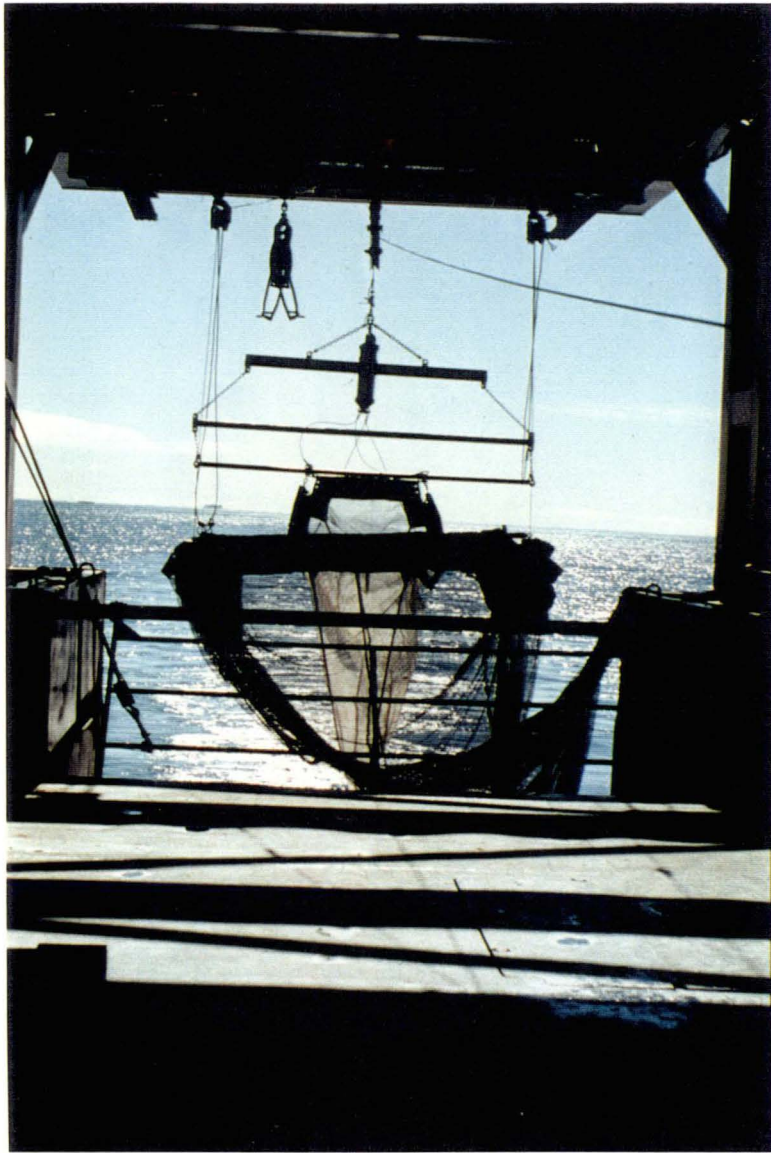
I. adult male (X 2.2); II. adult female (X 2.2); III. juvenile (X 2.2);
 IV. ventral view of male, (X 3.0); V. ventral view of female, (X 3.0).

Appendix 1. (a-e)



The development of the first petasma of *Euphausia superba* (X16).

a, undifferentiated endopod of juvenile; b, petasma of 2A male; c, petasma of 2B male; d petasma of 2C male; e, fully developed petasma of adult male. al, auxiliary lobe; il, inner lobe; ll, lateral lobe; ml, middle lobe; p, petasma; pa, additional process; pp, proximal process; pt, terminal process; sl, setigerous lobe; w, wing.



Appendix 2. Rectangular midwater trawl net (RMT 8). Mouth area 8 m² and mesh size 4.5 mm. Opening and closing achieved by electro-mechanical release activated via an electrically conducting towing wire (Williams et al. 1993).

Appendix 3. Fatty acid and sterol nomenclature.

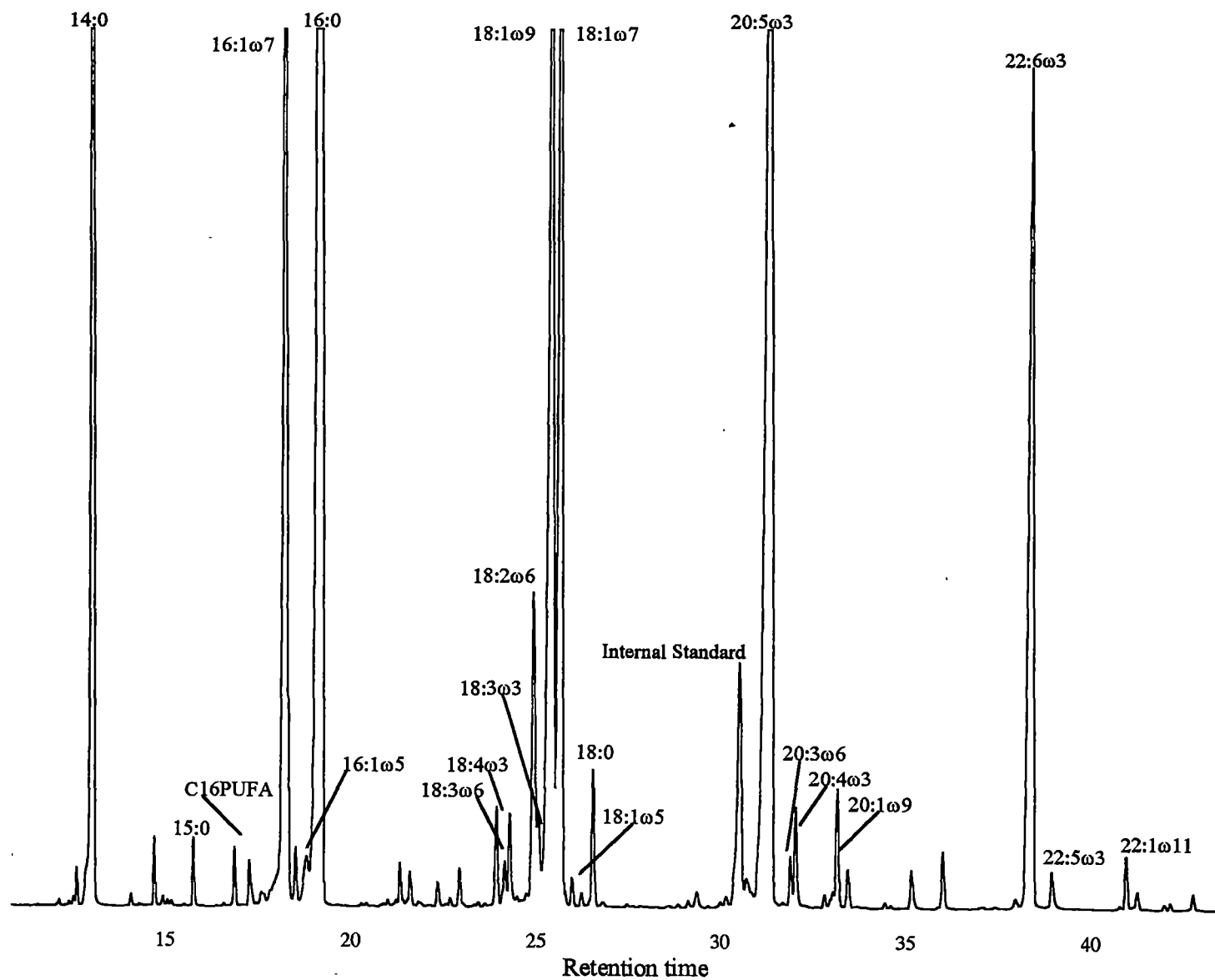
Fatty acids

Throughout this thesis fatty acids are named using the omega system. For example 20:4 ω 3 is equivalent to 20:4(n-3) where ω 3 and (n-3) indicate that the first double bond is located three carbon atoms from the terminal methyl group. In this example there are twenty carbon atoms in the molecule with four double bonds. Double bonds in polyunsaturated fatty acids are separated by methylene groups. Double bonds in the fatty acids are either in the *cis* (c) geometric configuration (alkyl groups on the same side of the double bond) or the *trans* (t) configuration (alkyl groups on opposing sides of the double bond). The double bonds of most fatty acids in nature are found in the *cis* configuration. The *cis* configuration is therefore assumed where configuration is undefined.

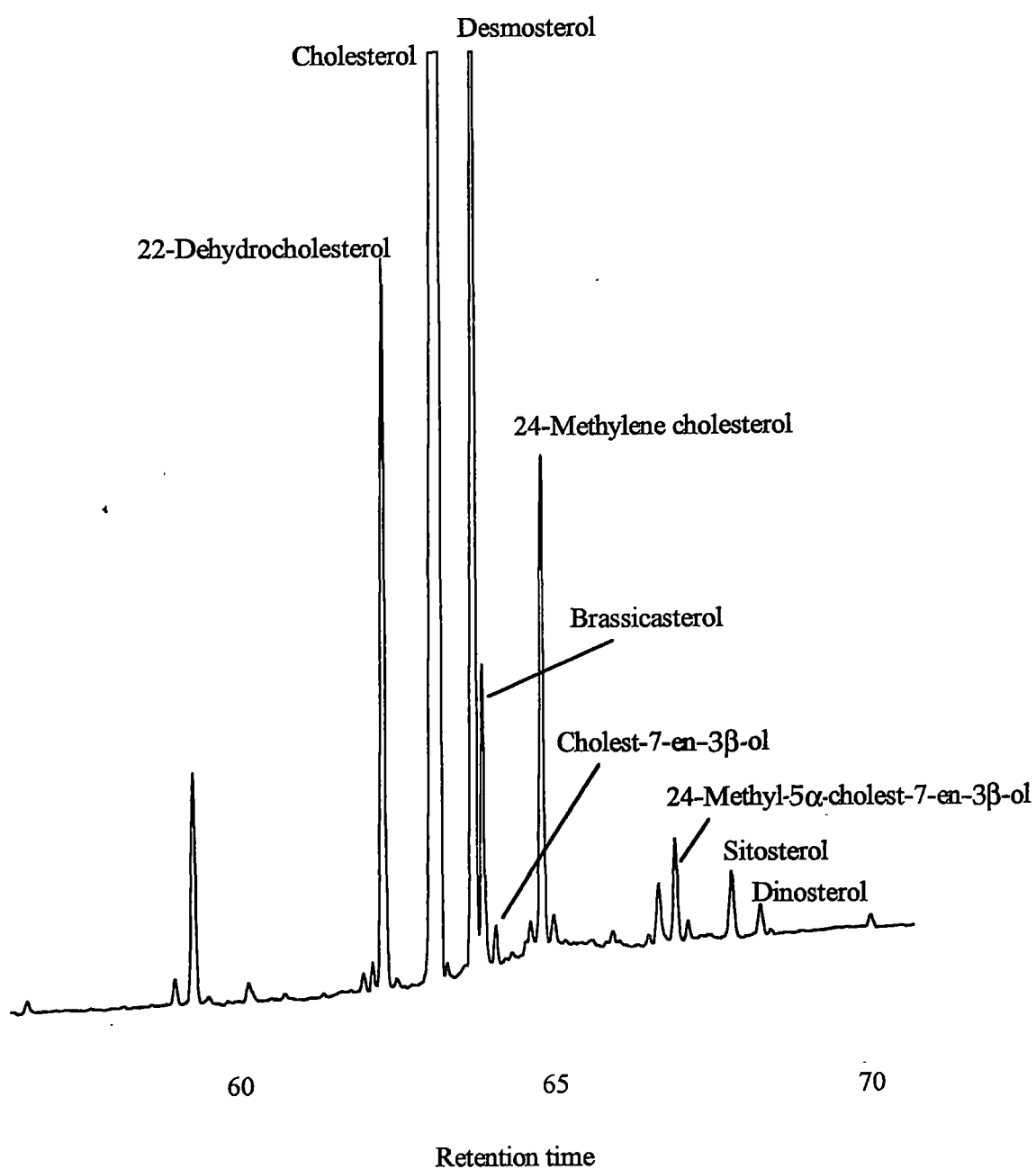
Sterols

Throughout this thesis sterols are described according to their structure. The common names as outlined below are used in the text. Stereochemistry at the C₂₄ position for 24-methylcholesta-5,22E-dien-3 β -ol and 24-ethylcholesta-5,22E-dien-3 β -ol (designated brassicasterol and stigmasterol respectively) and other C₂₄ alkylated sterols have not been determined.

<u>Common name</u>	<u>Full name</u>
trans-22-dehydrocholesterol	cholesta-5,22E-dien-3 β -ol
cholesterol	cholest-5-en-3 β -ol
desmosterol	cholesta-5,24-dien-3 β -ol
brassicasterol	24-methylcholesta-5,22E-dien-3 β -ol
24-methylene cholesterol	24-methylcholesta-5,24(28)-dien-3 β -ol
campesterol	24-methylcholest-5-en-3 β -ol
stigmasterol	24-ethylcholesta-5,22E-dien-3 β -ol
stigmastanol	24-ethyl-5 α -cholestan-3 β -ol
(iso) fucosterol	24-ethylcholesta-5,24(28)Z-dien-3 β -ol
dinosterol	4,23,24-trimethyl-5 α -cholest-22E-en-3 β -ol

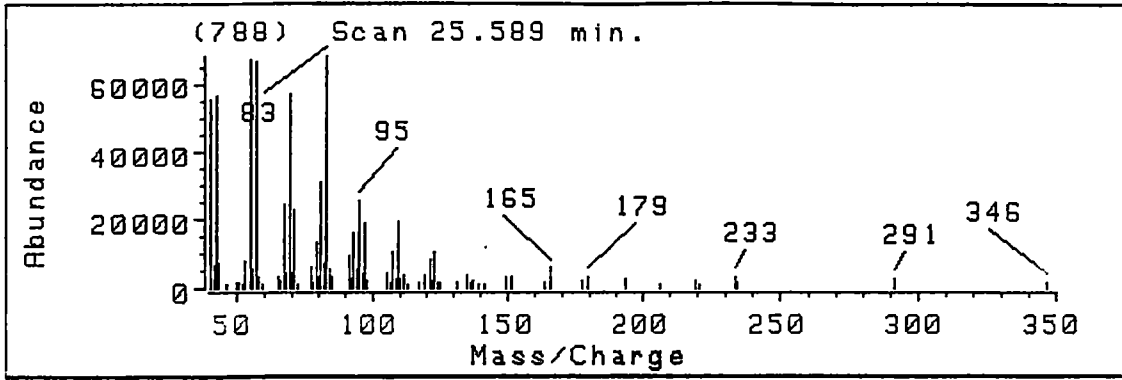


Appendix 4. Representative partial gas chromatogram of fatty acids found in *Eunhausia cuneirostris*

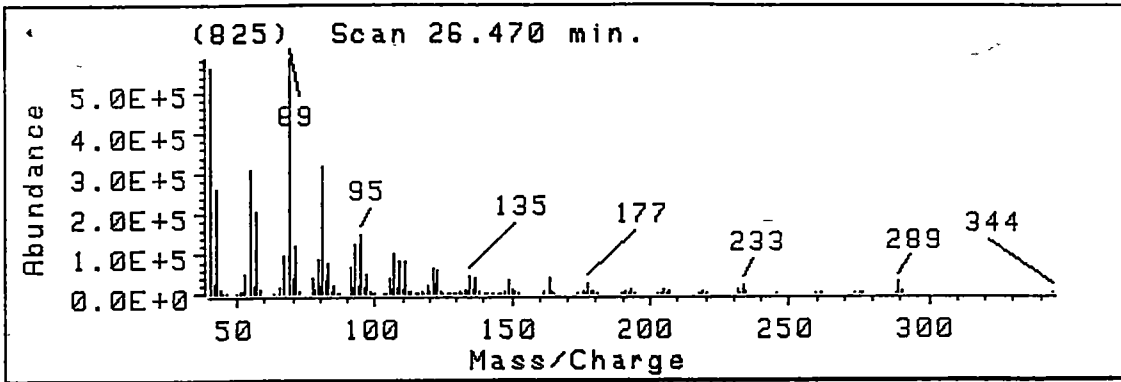


Appendix 5. Representative partial gas chromatogram showing sterols found in the digestive gland of *Euphausia superba*.

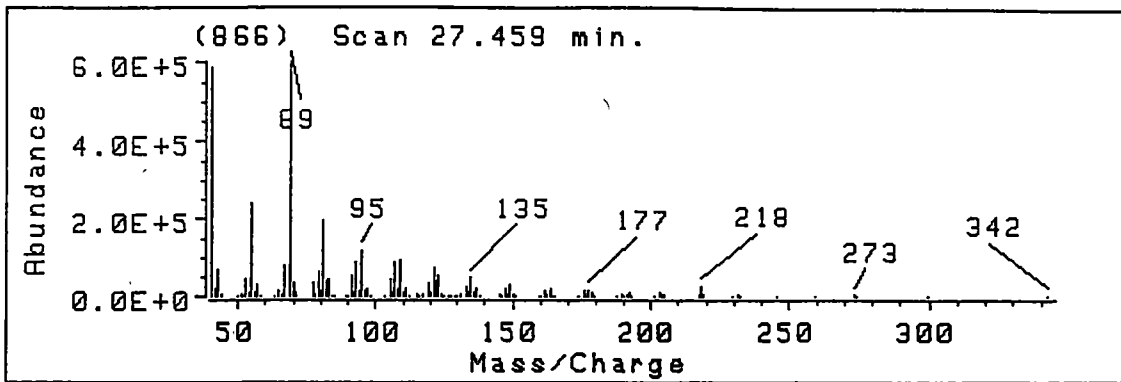
Isoprenoid C_{25:3}



Isoprenoid C_{25:4}



Isoprenoid C_{25:5}



Appendix 6. Mass spectral data for isoprenoid hydrocarbons found in the hydrocarbon fraction of the total lipids in *Euphausia superba*.